Nos. 2014-1374, 2014-1375

In the United States Court of Appeals for the Federal Circuit

ILLUMINA, INC.,

Appellant,

v.

LIFE TECHNOLOGIES CORPORATION,

Cross-Appellant.

Appeal from Decision of the United States Patent and Trademark Office's Patent Trial and Appeal Board on Inter Partes Re-Examination Application 95/000,529

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CERTIFICATE OF INTEREST

Counsel for Appellant Illumina, Inc. certifies the following information in compliance with Federal Rule of Appellate Procedure 26.1 and Federal Circuit Rules 26.1 and 47.4:

1. The full name of every party or amicus represented by us is:

   Illumina, Inc.

2. The names of the real parties in interest (if the party named in the caption is not the real party in interest) represented by us are:

   None

3. All parent corporations and any publicly held companies that own 10% or more of the stock of the party or amicus curiae represented by us are:

   None

4. The names of all law firms and the partners or associates that appeared for the party or amicus represented by us in the trial court or agency or are expected to appear in this Court are:

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Dated: June 23, 2014

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STATEMENT OF RELATED CASES

No appeal in or from this *inter partes* reexamination proceeding before the United States Patent and Trademark Office Patent Trial and Appeal Board was previously before this or any other appellate court.

U.S. Patent No. 6,831,994 was the subject of litigation in *Life Technologies Corp., et al. v. Illumina, Inc., et al.*, No. 09-cv-00706-BMS (D. Del.) filed September 21, 2009, and subsequently transferred to the United States District Court for the Southern District of California. *See Life Technologies Corp., et al. v. Illumina, Inc., et al.*, No. 11-cv-00703-BTM (S.D. Ca.). On January 16, 2013, the district court entered an order staying litigation of the claim(s) involving U.S. Patent No. 6,831,994 pending completion of the *inter partes* reexamination. *See id.*, Dkt. No. 431.

The district court litigation also includes U.S. Patent Nos. 6,654,505 and 7,232,656. Life Tech also filed requests for *inter partes* reexamination of these patents. And the district court likewise entered an order staying litigation of the claims involving U.S. Patent Nos. 6,654,505 and 7,232,656 pending completion of the *inter partes* reexamination proceedings. *See id.*, Dkt. No. 431. The reexamination of U.S. Patent No. 6,654,505 is the subject of a separate appeal before this Court, *see Life Technologies Corp. v. Illumina, Inc.*, Nos. 14-1215, 14-

1216 (Fed. Cir.), while U.S. Patent No. 7,232,656 remains before the Patent Trial and Appeal Board.

In addition, the district court litigation includes allegations by Life Tech that Illumina infringes three patents exclusively licensed to Life Tech. By opinion and order dated March 20, 2013, the District Court granted Illumina's motion for summary judgment of non-infringement of the Life Tech Patents. *See Life Technologies Corp.*, No. 11-cv-00703-BTM, Dkt. No. 488. The Court entered final judgment of non-infringement on April 28, 2014, *see id.*, Dkt. No. 526, and Life Tech filed a notice of appeal on May 27, 2014, *see id.*, Dkt. No. 531. This Court docketed the appeal on June 2, 2014. *See Life Technologies Corp., et al. v. Illumina, Inc.*, No. 14-1513 (Fed. Cir.).

JURISDICTIONAL STATEMENT

Appellant Illumina, Inc. ("Illumina") appeals the decision of the United States Patent and Trademark Office's ("USPTO") Patent Trial and Appeal Board ("PTAB") rejecting claims 1-5 of U.S. Patent No. 6,831,994 (the '994 Patent) as anticipated and/or obvious. The PTAB had jurisdiction to review the adverse decisions of the Examiner. *See* 35 U.S.C. §§ 6(b), 134, and 315.

On December 30, 2013, Illumina timely appealed from the PTAB's February 26, 2013 Decision on Appeal and October 29, 2013 Decision on Rehearing by filing a Notice of Appeal with the USPTO. The PTAB's judgment in the *inter partes* reexamination is final, and the Court of Appeals for the Federal Circuit has jurisdiction over the present appeal pursuant to 28 U.S.C. § 1295(a)(4)(A).

STATEMENT OF THE ISSUES ON ILLUMINA'S APPEAL

1. Did the PTAB err in concluding that claim 1 of the '994 Patent is not limited by the claim's preamble that provides, in conjunction with the specification and prosecution history, the antecedent basis for, and definition of, the claim's remaining limitations?

2. Did the PTAB err in entering new rejections of claims 1-5 of the '994 Patent based on the PTAB's erroneous construction of the preamble in independent claim 1?

INTRODUCTION

Illumina is a leading developer, manufacturer, and marketer of life science tools and integrated systems for the analysis of genetic variation and function. Among other things, Illumina manufactures and markets products incorporating innovative techniques for the analysis of complex chemical or biological systems, including DNA sequencing tools. Illumina protects its innovations through the patent process.

The ’994 Patent, at issue in this matter, protects a “system for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps . . . .” ’994 Patent, claim 1 (A69). The instant *inter partes* reexamination proceeding, which Life Technologies Corporation (“Life Tech”) instituted from a more comprehensive district court lawsuit between the parties, turns on this simple but significant language in the preamble of representative claim 1 of the ’994 Patent that has resulted in opposing claim constructions—and resulting conclusions on patentability—within the USPTO.

On the one hand, the Examiner found that the phrase “during a sequence of processing steps” further limits the ’994 Patent’s first claim drawn to the connected phrase, “a sequence of optical signals.” On the other hand, the PTAB concluded that the preamble phrase “during a sequence of processing steps” identifies only an environment in which the claimed system may operate. Based on the latter

conclusion, the PTAB rejected claims 1-5 of the ’994 Patent as anticipated and/or obvious.

Because the PTAB’s conclusion ignores, *inter alia*, (1) the antecedent connection between the preamble’s recitation of a “sequence of processing steps” and the key element of a “sequence of optical signals;” (2) the problems in microparticle analysis addressed by the ’994 Patent, and (3) reliance on the preamble language to overcome a prior art rejection of claim 1 during initial prosecution of the ’994 Patent, and is therefore contrary to the entire record of the ’994 Patent, the PTAB’s construction of claim 1’s preamble is unreasonable. The PTAB’s resulting rejections of claims 1-5 should be overturned.

STATEMENT OF THE CASE

On September 21, 2009, Life Tech filed suit in the United States District Court for the District of Delaware, alleging that Illumina’s Genome Analyzer® DNA-sequencing system infringes various claims of three patents exclusively licensed to Life Tech. After Illumina filed counterclaims alleging infringement by Life Tech of various claims of four Illumina patents, including the ’994 Patent, Life Tech promptly filed the instant request for *inter partes* reexamination of the ’994 Patent. Life Tech asserted sixty-six anticipation and obviousness challenges against original claims 1-4 of the ’994 Patent based on sixteen prior art references.

The Examiner initially adopted four of Life Tech's proposed rejections. Illumina responded by amending the '994 Patent to add claims 5-35—which include all of the limitations of claim 1 as well as additional limitations—and explaining that prior art references cited by the Examiner involve analysis of particles in a fixed environment and therefore do not meet the requirement in '994 claim 1 that optical signals are detected "during a sequence of processing steps." *See* A97–100, 103.

In a December 15, 2010 Action Closing Prosecution, the Examiner agreed with Illumina that the preamble phrase "has patentable weight" because it "provides antecedent basis for an important element in the claim." A195. Finding that certain prior art references did not disclose the preamble limitation, the Examiner thus withdrew rejections of claims 1-4 based on those references. *See* A201–02, 205–06, 210–12. The Examiner nevertheless rejected claims 1-35 of the '994 Patent as anticipated, obvious, or improperly enlarging the scope of the claimed invention. *See* A179–97.[1] The Examiner based the anticipation and obviousness rejections, in large part, on one reference, Sydney Brenner's PCT publication WO 96/12014. *See* A179–97.

Illumina again amended the '994 Patent, altering the language in claims 29–35, and substantively challenged the Examiner's rejections (including with the

[1] These rejections are likely to be the principal subject of Life Tech's cross-appeal. *See infra* Section C.2.

assistance of an expert, Dr. Jeff Gelles). The Examiner then concluded proceedings with a Right of Appeal Notice that (1) maintained the rejections of claims 1-24, 26-30, 33, and 34 as anticipated or obvious (largely based on Brenner); (2) maintained the withdrawal of rejections to claims 1-4 based on the preamble limitations; (3) withdrew the rejection of claims 29-31 and 33-35 for improperly enlarging the claimed invention based on Illumina's amendment of those claims; and (4) withdrew rejections of claims 25, 31, and 35 by adopting Illumina's construction of "closely packed" microparticles.

Illumina and Life Tech cross-appealed to the PTAB. On February 26, 2013, the PTAB issued a Decision on Appeal reversing the Examiner's rejections of claims 1-24, 26-30, and 33-34 as anticipated or obvious over references centered on Brenner, but reinstated the previously withdrawn rejections of claims 1-4 (and added a rejection of claim 5) based on its conclusion "that the Examiner erred in interpreting" the preamble to claim 1 of the '994 Patent. *See* A22. In the PTAB's view, the preamble lacks patentable weight because it "does not further limit or define" the claim, A24–25, or recite any component to perform the preamble's "sequence of processing steps," *see* A25.

Having reversed the Examiner's construction, the PTAB reinstated the previously withdrawn anticipation and obviousness rejections of claims 1-4 (and added a rejection of dependent claim 5). The PTAB reiterated its construction in

denying Illumina's request for rehearing. This appeal followed.[2] The overarching question on Illumina's appeal is whether the preamble phrase "during a sequence of processing steps" further limits independent claim 1 of the '994 Patent.

STATEMENT OF THE FACTS

A. The '994 Patent.

The '994 Patent issued on December 14, 2004 from an application filed July 17, 2001. A44. The '994 Patent claims priority from an application filed May 22, 1998. A44.[3]

1. The '994 Specification.

The '994 Patent is entitled "System and Apparatus for Sequential Processing of Analytes." A44.[4] In brief, the invention involves attachment of analytes to microparticles thereafter disposed "inside of a flow chamber where steps of an analytical process are carried out by delivering a sequence of processing reagents to the microparticles by a fluidic system under microprocessor control." '994 Patent, Abstract (A44). "In response to such process steps, an optical signal is generated at the surface of each microparticle," recorded, and analyzed. *Id*. (A44).

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[2] Life Tech has separately cross-appealed, among other things, the grounds for rejection reversed by the PTAB. Illumina will further address those grounds in response to Life Tech's principal Cross-Appellant's Brief.

[3] The listed inventors include John Bridgham, Kevin Corcoran, George Golda, Michael C. Pallas, and Sydney Brenner. A44.

[4] An "analyte" is any substance that is the subject of analysis. *See, e.g.*, Merriam-Webster Online Dictionary, *available at* http://www.merriam-webster.com/dictionary/analyte.

To foster analysis, "[a] key feature of the invention is the correlation of the sequence of optical signals generated by each microparticle in the planar array during the analytical process." *Id*. (A44).

More generally, the invention relates to "systems and apparatus for monitoring and carrying out reactions on arrays of microparticles." *Id*., 1:13–15 (A56). In particular, the invention is designed to facilitate "analytical techniques that employ parallelization and miniaturization of analyte processing," *id*., 1:18–20 (A56), "to understand and analyze complex chemical and biological systems," *id*., 1:17–18 (A56). While "microparticles generally facilitate the construction and manipulation of large repertoires of analytes . . . ," *id*., 1:37–39 (A56), "handling and manipulating large numbers of microparticles, e.g. tens to hundreds of thousands, . . . gives rise to many difficulties" in analyzing complex chemical and biological systems, *id*., 1:40–43 (A56). These difficulties include "how to track individual microparticles through multiple steps of a process, . . . the ability to uniformly deliver reagents to microparticles for carrying out steps of an analytical process," and various "disruption[s]" relating to delivery of processing reagents to the microparticles—and the analytes attached thereto—being studied. *Id*., 1:43–56 (A56).

In response, the '994 Patent is directed to a "system and apparatus [that] permit[s] the tracking and analysis of multiple analytes anchored to separate

microparticles through a sequence of several processing and/or analysis steps." *Id*., 1:60–64 (A56). The resulting invention solves the recited processing difficulties with:

> [A]n apparatus comprising a flow chamber for disposing a population of microparticles in a planar array; fluidic means for sequentially delivering processing reagents from one or more reagent reservoirs to the flow chamber; and detection means for detecting a sequence of optical signals from each of the microparticles of the population.

Id., 2:20–26 (A56).

The '994 Patent discloses "a schematic representation" of an apparatus with such flow chamber and detection system for use in analyzing microparticles at Figure 1*a*. *Id*., 2:60–63 (A56).

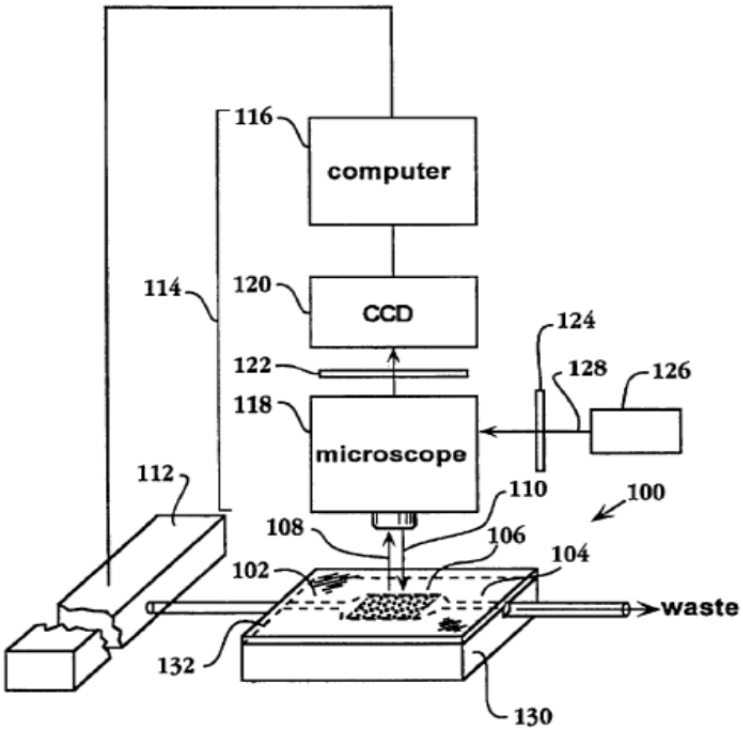

Fig. 1A

Id., Figure 1*a* (A46). This schematic embodiment "for detecting fluorescent signals" includes a flow chamber that "holds microparticles in a planar array from which optical signals (108) generated by analytes and/or reactants on microparticles can be collected and imaged," all under the control of a computer. *See id*., 4:65 through 5:6 (A57–58).

The '994 Patent further discloses schematics and illustrations of a "flow chamber," *id*., 2:64 through 3:6 (A56–57); *see also id*., Figures 1*b*, 2*a* through 2*c*, 3*a* through 3*d*, 4 (A47–51); and a "fluidics system," *id*., 3:7–8 (A57); *see also id*., Figure 5 (A52), connected to the flow chamber for conducting a series of processing steps. Indeed, "[a] key feature of ***the invention*** is flow chamber (100)," *id*., 5:23 (A58 (emphasis added)), which "is operationally associated with fluidic system (112) and detection system (114) . . .," *id*., 5:4–5:6 (A58). *See also id*., 8:4–7 (A59) ("Preferably, process reagents are delivered to flow chamber (100) by the fluidic system illustrated in [Figure 5] which has the capacity to handle many different reagents for complex analytical processes."). In turn, "[k]ey functions of the flow chamber include . . . holding a population of microparticles in a substantially immobilized planar array . . . during a sequence of processing steps . . . ." *Id*., 5:37–39 (A58).

The ’994 Patent further associates a flow chamber and fluidics system within “a flow chart summarizing operation of the system of the invention,” *id*., 3:15–16 (A57),

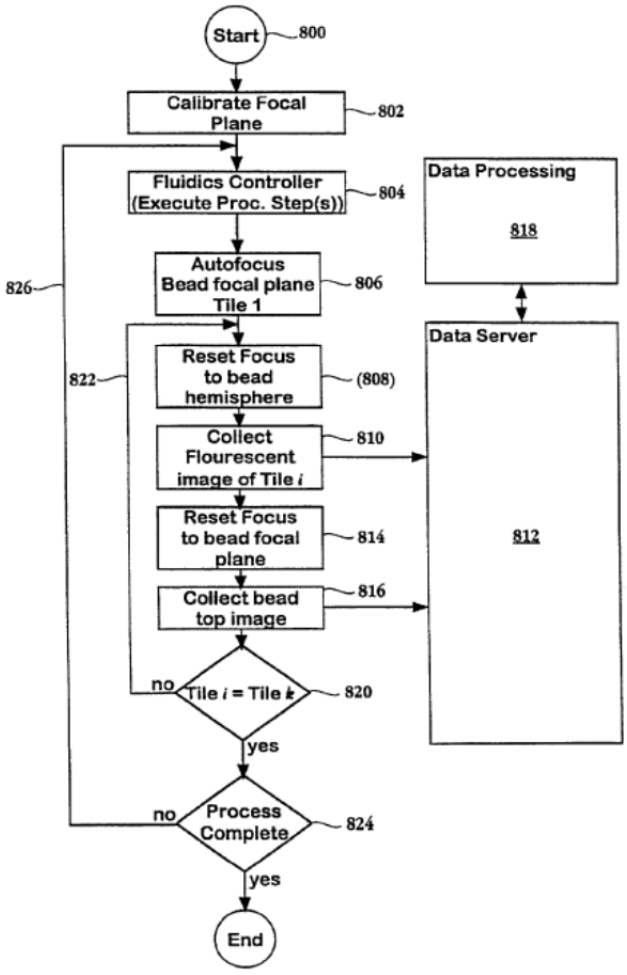

Fig. 8

id., Figure 8 (A55), in conducting a series of “process steps,” *id*., 9:59 (A60), in which optical signals are collected from microparticles, *id*., 10:28 (A60). *See also id*., 9:32–33 (A60) (describing Figure 8 as a summation of “[t]he general operation of the system of the preferred embodiment”); *id*., 19:65 through 20:67 (A65) (describing series of process steps, with successive imaging, in cDNA sequencing).

The invention's various aspects incorporate the apparatus to detect "[o]ptical signals generated by, or produced as a result of, the interaction of processing reagents and [analytes] on the microparticles . . . ." *Id*., 2:48–51 (A56). *See also id*., 8:48–50 (A59) ("An important feature of detection means (114) of the invention is the ability to keep track of individual microparticles through multiple process steps and/or cycles."). For example, "[p]referably, the sequences of optical signals are generated as a result of a multi-step analytical process, such as nucleic acid sequence analysis." *Id*., 2:26–29 (A56). In this example, "[p]olynucleotides loaded onto microparticles may be simultaneously sequenced in the instant apparatus using a 'base-by base' DNA sequencing methodology," *id*., 15:3–5 (A63), such as the use of "[e]ncoded adaptors" through "repeated cycles of ligation, identification, and cleavage," *id*., 15:33–42 (A63). "In the identification step, successive sets of tag complements are specifically hybridized to the respective tags carried by encoded adaptors ligated to the ends of the target polynucleotides . . .," *id*. 15:54–57 (A63), by successively passing a series of fluids through the flow chamber, *see id*., 19:61 through 20:67 (A65). And identification is carried out through optical signals generated by successively exciting fluorescent labels attached to the encoded adaptors. *See id*. (A65).

2. The Disputed ’994 Claims.

At the time of issuance, the ’994 Patent included four claims. Independent, and representative, claim 1 provides (terms underlying Illumina’s appeal emphasized):

> A system ***for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps***, the system comprising:
>
> a planar array of uniformly sized spherical microparticles, wherein the coefficient of variation of the diameters of said microparticles is less than five percent;
>
> an optical train effective to collect and focus ***the sequence of optical signals*** from the microparticles, and to record at least one optical characteristic of each microparticle which can be used to determine the approximate center of said microparticle;
>
> an imaging device onto which ***said signals*** are focused, effective to generate and record a sequence of digital images of the microparticles, with sufficient resolution for individual microparticles to be distinguished; and
>
> signal tracking means effective to correlate ***the optical signals*** from each of the microparticles in each of the sequence of digital images with said center of said microparticle.

’994 Patent, claim 1 (A69) (emphases added).

During the *inter partes* reexamination proceedings, Illumina amended the ’994 Patent to include claims 5-35, which include all of the limitations of claim 1 as well as additional limitations. *Compare id.* (A69) *with* A97–100.

B. Prosecution of the ’994 Patent.

Initially filed July 17, 2001, the Examiner rejected the application for the ’994 Patent in an Office Action dated June 24, 2003. *See* A528. At the time of rejection, claim 1 of the application read:

> A detection apparatus for detecting a sequence of optical signals from each of a plurality of microparticles comprising:
>
> an optical train effective to collect and focus the sequence of optical signals from the microparticles, and to record at least one optical characteristic of each microparticle which can be used to determine the approximate center of said microparticle;
>
> an imaging device onto which said signals are focused, effective to generate and record a sequence of digital images of the microparticles, with sufficient resolution for individual microparticles to be distinguished; and
>
> signal tracking means effective to correlate the optical signals from each of the microparticles in each of the sequence of digital images with the position of said microparticle.

A529.[5] In the Examiner’s view, U.S. Patent No. 4,125,828 (“Resnick”) rendered this claim obvious by disclosing, *inter alia*, “a system ‘comprising signal tracking means for correlating the optical signals from each particle in each of the digital images with the position of the particle.’” *See* A530, 533 (quoting Office Action, emphasis omitted)).

[5] At the time, current claim 1 of the ’994 Patent was numbered as claim 8. As part of the request for continued examination, the applicant cancelled preceding claims 1-7, and re-numbered the remaining original claims to their present format. *See* A529.

To overcome this rejection, the applicant amended the preamble to claim 1 (deletions indicated by strikethrough and insertions indicated by underline):

> A ~~detection apparatus~~ system for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps, the system comprising . . . .

A529. In addition to this sequence amendment to the preamble, the applicant further amended claim 1 to include:

> a planar array of uniformly sized spherical microparticles, wherein the coefficient of variation of the diameters of said microparticles is less than five percent . . . .

A529.

Accompanying these amendments, the applicant argued that the amended system of the invention "allows the array of microparticles to be tracked during a sequence of processing steps, as described above." A533. Contrary to the detection of optical signals during a sequence of processing steps as recited in the amended claim 1, Resnick involves study of a sample that is "fixed" prior to analysis. A533 (citing U.S. Patent No. 4,125,828 at 7:1–10). Accordingly, the applicant argued, "there is no teaching or suggestion in Resnick" of a system for tracking a sequence of images through a sequence of processing steps, and the obviousness rejection of claim 1 must be withdrawn. A533–34.[6]

[6] The second amendment to claim 1—limiting the claimed microparticles—responded to a separate part of the Examiner's rejection based on Resnick, *see* A532, and a second prior art reference, U.S. Patent No. 5,578,832 ("Trulson"), *see*

In response to these amendments and arguments, the Examiner withdrew the rejection of claim 1, and the '994 Patent issued with its original four claims.

C. The *Inter Partes* Reexamination Proceedings Before the USPTO.

1. Proceedings Before the Examiner.

On February 19, 2010, Life Tech requested an *inter partes* reexamination of claims 1-4 of the '994 Patent. *See* A71.[7] Spanning more than four-hundred pages, Life Tech's request asserted sixty-six anticipation and obviousness challenges to claims 1-4 of the '994 Patent, based on sixteen prior art references (either alone or in various combinations). *See* A77–93. On May 10, 2010, the Examiner issued a Non-Final Office Action adopting four of Life Tech's proposed rejections of claims 1-4. *See* A77–83.

Illumina responded to the Office Action by substantively contesting the Examiner's findings of anticipation and obviousness, and amending the '994 Patent to add claims 5-35, which include all of the limitations of claim 1 (as well as additional limitations). *See* A97–100. In particular, Illumina explained that a number of the Examiner's rejections of claims 1-4 relied on prior art references

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A530, 531–32. The Examiner acknowledged that Trulson related solely to the limitations on the size and shape of the microparticles, and not to the tracking of a sequence of images. *See* A532, 533.

[7] Although Life Tech initially filed its request for *inter partes* reexamination on January 29, 2010, *see* A71, that request was rejected for failure to comply with filing requirements, *see* A71. The initiation of the *inter partes* reexamination is therefore based upon the filing date of Life Tech's Replacement Request for *Inter Partes* Reexamination.

that, among other deficiencies, are "directed to tracking cellular movement on a stable, unchanging microscope slide,"[8] and therefore lack disclosures relating to detection of optical signals " 'during a sequence of processing steps' as provided . . . [in the preamble of] claim 1 of the '994 patent." A103. For its part, Life Tech disputed that the claim 1 preamble limits the '994 Patent, contended that the prior art references nevertheless disclose the preamble's "sequence of processing steps" limitation, and challenged the patentability of new claims 5-35 as anticipated, obvious, or lacking adequate enablement. *See* A124–71.

On December 15, 2010, the Examiner issued an Action Closing Prosecution rejecting claims 1-35 of the '994 Patent as anticipated, obvious, or improperly enlarging the scope of the claimed invention. *See* A179–97. The Examiner based the anticipation and obviousness rejections, in large part, on one reference, Sydney Brenner's PCT publication WO 96/12014 ("Brenner"). *See* A179–97.[9]

Indeed, the Examiner withdrew the prior rejections of claims 1-4 based on Lee, *see* A201–02, Schmidt, *see* A205–06, and Gelles, *see* A210–12. In issuing these withdrawals, the Examiner found that the "phrase 'a sequence of optical

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[8] The relevant references are (1) Lee, *et al.*, U.S. Patent No. 6,057,150 ("Lee") (A477); (2) Schmidt, *et al.*, "Integrin-Cytoskeletal Interactions in Migrating Fibroblasts are Dynamic, Asymmetric, and Regulated" ("Schmidt") (A502); and (3) Gelles, *et al.*, "Tracking kinesin-driven movements with nanometre-scale precision ("Gelles") (A517). *See generally* A107–17.

[9] These rejections, and the Brenner reference, are likely to be the principal subject of Life Tech's cross-appeal. *See infra* Section C.2.

signals from each of a plurality of microparticles during a sequence of processing steps' recited in the preamble of the claims has patentable weight." A195. The Examiner explained that the preamble language "provides antecedent basis for an important element in the claim" and "[i]ndependent claim 1 refers back to this element in the preamble several times," which "[t]hus . . . gives life and breath to the body of the claim." A195.[10] Because the Examiner concluded that Lee, *see* A201–02, Schmidt, *see* A205–06, and Gelles, *see* A210–12, fail to disclose the limitations of the preamble, the Examiner withdrew the rejections.

Illumina responded to the Examiner's further rejection of claims 1-35 by amending claims 29-35, *see* A230–31,[11] substantively contesting the Examiner's application of Brenner, *see* A231–46, and offering in support the Declaration of Dr. Jeff Gelles ("Gelles Decl."), *see*, *e.g.*, A230. Dr. Gelles both explained the differences between the '994 Patent and the references relied upon by the Examiner, *see* Gelles Decl. ¶¶ 24–82 (A568–75), and further explained the invention disclosed in the '994 Patent.

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[10] The Examiner further found, in light of the '994 Patent's specification, *see infra*, that the phrase " 'processing steps' has a particular meaning, namely, steps associated with the processing of microparticles in a fluidic system (e.g., exposing the microparticles to various reagents . . .)" that defines "a particular part of the operation of the system distinct from other steps." A195–96 (citing '994 Patent, Figure 8, step 804 and 8:4–40, 9:61–65).

[11] Illumina canceled claim 32. *See* A226.

Among other things, Dr. Gelles explained that "[t]he meaning of the complete phrase 'during a sequence of processing steps' is made apparent in the specification" to indicate "manipulating the environment containing microparticles, where the manipulation includes the addition of reagents and washes to a flow chamber using a fluidics controller." Gelles Decl. ¶ 13 (A566). *See also id*. ¶¶ 14–23 (A566–67) (citing '994 Patent, 5:23–42, 8:4–5, 9:59–61, 10:24–25, 9:59 through 10:27, and Figure 8). Indeed, the "process steps are initiated" by the fluidics controller. *See id*. ¶ 17 (A567) (quoting '994 Patent, 9:59–61); *see also id*. ¶ 18 (A567).

Over Illumina's arguments and evidence, the Examiner maintained the rejections of claims 1-24, 26-30, 33, and 34 as anticipated or obvious (again, largely based on Brenner). *See* A280–84. The Examiner similarly rejected Life Tech's challenge, *see* A263–69, to the Examiner's conclusion that claim 1's preamble is limiting, and thus maintained the withdrawal of rejections to claims 1-4 based on Lee, Schmidt, and Gelles, *see* A288–89. The Examiner reiterated,

> The phrase, 'during a sequence of processing steps,' is part of the description of the 'sequence of optical signals' . . . . This *sequence of optical signals* is recited in the body of the claim several times and therefore, the language of the preamble gives life and breath to the body of the claim.
>
> In other words, in light of the ['994 Patent] specification, the preamble of claim 1 is read as 'a system for detecting a sequence of optical signals' wherein the detected sequence of optical signals is specifically 'a sequence of optical signals from each of a plurality of

> microparticles during a sequence of processing steps.' The preamble is *not* read as 'a system for detecting a sequence of optical signals from each of a plurality of microparticles' (end of phrase) wherein the detecting merely occurs during a sequence of (generic) processing steps.

A288 (emphases original).

In addition to maintaining the withdrawals based on the preamble, the Examiner withdrew the rejection of claims 29-31 and 33-35 for improperly enlarging the claimed invention based on Illumina's amendment of those claims after the Action Closing Prosecution. *See* A284. The Examiner also withdrew rejections of claims 25, 31, and 35 by adopting Illumina's construction of "closely packed" microparticles in light of the '994 specification and the understanding of a skilled artisan as detailed in the Gelles Declaration. *See* A284.

2. Proceedings Before the Patent Trial and Appeal Board.

Life Tech timely appealed the Examiner's allowance of claims 25, 31 and 35, and refusal to adopt additional rejections—including the withdrawn rejections of claims 1-4 based on Lee, Schmidt, and Gelles—to the PTAB. *See* A71; A336. Illumina cross-appealed the Examiner's rejections of claims 1-24, 26-30, and 33-34. *See* A71; A298–311. On February 26, 2013, the PTAB reversed the Examiner's rejections of claims 1-24, 26-30, and 33-34 as anticipated or obvious over references centered on Brenner, but reinstated the rejections of claims 1-4

(and added a rejection of dependent claim 5) over Lee and Gelles as new grounds for rejection pursuant to 37 C.F.R. § 41.77(a). *See* A31–35.

With respect to the new grounds for rejection,[12] the PTAB concluded "that the Examiner erred in interpreting" claim 1 of the '994 Patent. A22. In so holding, the PTAB reviewed three of the limitations in claim 1—the claimed "optical train," "imaging device," and "signal tracking means"—and concluded that each describes "functions [that] are express limitations that constrain the . . . [claimed limitations] to structures which are able to perform the recited functions." A24.[13] The PTAB then concluded that the preamble "does not further limit or define" the structures in the three claimed limitations, "but rather simply reflects the intention that the system is used during such processing steps." A24–25. Finally, the PTAB explained, "there is no component recited in the body of the claim which would perform the sequence of processing steps," in contrast to "claim 29 which has similar language but adds the additional limitation of 'a fluidics controller effective to deliver reagents to the flow cell for a sequence of processing steps.'" A25.

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[12] Illumina limits its discussion of the PTAB decision to the grounds underlying Illumina's appeal. Because the PTAB's decisions to reverse the Examiner's rejections of claims 1-24, 26-30, and 33-34, *see* A6–20, and to not adopt certain additional rejections proposed by Life Tech, *see, e.g.*, A21–22, 28–31, form the likely basis of Life Tech's cross-appeal, Illumina reserves discussion of such PTAB decisions until its response to Life Tech's principal brief on cross-appeal.

[13] The PTAB did not consider the limitation relating to the size and shape of microparticles in the planar array. *See* '994 Patent, claim 1 (A69).

Having reversed the Examiner's construction of claim 1, the PTAB concluded that the Examiner erred in withdrawing the rejection of claims 1-4—and in not further rejecting claim 5—as anticipated or obvious over Lee and Gelles. *See* A25, 26–28.[14] Because it found that the preamble does not limit claim 1 of the '994 Patent, the PTAB did not address Life Tech's alternative argument that Lee, Schmidt, and/or Gelles disclose a "sequence of processing steps" as recited in the preamble and specification. *See* A354–358.

In response to Illumina's request for rehearing, *see* A454, the PTAB acknowledged that its rejections of claims 1-5 rested solely "upon [the Board's] finding that the Examiner erred in interpreting . . . the preamble of claim 1 . . . ." A38. The PTAB likewise acknowledged that "the '994 Patent teaches that the claimed system was designed to handle microparticles through a series of processing steps and collect optical signals from microparticles during these steps." A39. Indeed, the PTAB conceded that "[a] system is described in the '994 Patent to include an apparatus (which comprises a flow chamber, fluidic means, and detection means) . . .," A40, and which is responsible for the processing steps.

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[14] The PTAB's rejection of claim 5 as obvious further relies on U.S. Patent No. 5,589,401 ("Hansen") in combination with Lee and Gelles. *See* A27–28.

In contrast, the PTAB found that the Examiner properly withdrew rejections of claims 1, 3, 4, and 5 as anticipated by Schmidt (or obvious in view of Schmidt) because Schmidt fails to disclose microparticles or a planar array as required by claim 1. *See* A25–26, 28.

The PTAB nevertheless reiterated its view that the preamble provides no further limitation to claim 1 because "the limitation that the optical signals are detected in a 'sequence of processing steps' has no corresponding structure in the claim." A40. Rather, the PTAB concluded, without citation, that it could not properly "insert a method step into a system claim," A40, "or . . . import a structure in the system for performing the processing steps," A41. The PTAB accordingly denied Illumina's request for rehearing, *see* A42, and the parties timely appealed.

SUMMARY OF THE ARGUMENT

Illumina's appeal turns on a common question: whether the preamble to a claim provides an additional limitation. To answer that question, this Court—and the Board before it—must "examin[e] . . . the entire patent record to determine what invention the patentee intended to define and protect." *Rowe v. Dror*, 112 F.3d 473, 478 (Fed. Cir. 1997).

1. In concluding that the preamble to claim 1 of the '994 Patent does not further limit the claim, the PTAB either ignored or misunderstood the entire record of the '994 Patent. Rather, in light of the '994 Patent's full record—its claims, specification, and prosecution history—the PTAB's largely conclusory construction is unreasonable.

At the outset, the PTAB's principal conclusion that the preamble does not "limit or define" the succeeding limitations of '994 claim 1 fails to recognize the

antecedent relationship between the two halves of the preamble phrase. In full, the preamble recites that the claimed system is "for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps." '994 Patent, claim 1 (A69). While the remainder of claim 1 constantly refers to the "sequence of optical signals" recited in the preamble, that key phrase is itself dependent on its twin provision in the preamble of a "sequence of processing steps," which generates the optical signals. Absent the antecedent reference to "processing steps" in the preamble, the subsequent limitation description of a "sequence of optical signals" is little more than "empty language." *Griffin v. Bertina*, 285 F.3d 1029, 1033 (Fed. Cir. 2002). The PTAB's resort to conclusory labeling of claims as "method" or "system" does not salvage its failure to recognize this antecedent relationship, or relieve it of the obligation to determine what the preamble language means in light of the entire patent record.

Moreover, the '994 Patent specification confirms the connection between the "sequence of processing steps" and the resulting "sequence of optical signals." The specification further defines both the problems in analyte processing that the claimed invention sets out to solve, and the structure of an apparatus and components of incorporated processing steps that solve those problems in relation to "a sequence of processing steps." By thus including "a sequence of processing steps" within the "essence of the invention," *MEMS Tech. Berhad v. ITC*, 447 F.

App'x 142, 154 (Fed. Cir. 2011), the specification again confirms the limiting nature of claim 1's preamble.

Finally, the '994 Patent's prosecution history demonstrates that the applicant amended claim 1 with the disputed preamble language in order to overcome an initial obviousness objection. Following the applicant's argument that the amended language "allows the array of microparticles to be tracked during a sequence of processing steps," the Examiner allowed claims 1-4 of the '994 Patent. Such reliance in prosecution provides additional assurance that the preamble language is limiting.

Viewed in this proper context, the PTAB's construction of the disputed preamble language is contrary to the entire record of the '994 Patent and thus unreasonable.

2. Because the PTAB's rejection of claims 1-5 of the '994 Patent rested solely on its erroneous construction of the preamble, the PTAB's anticipation and obviousness rejections must be rejected in turn. The PTAB's failure to make any factual findings regarding the content of the prior art supports vacatur and remand to the PTAB to make findings commensurate with the factual questions underlying the doctrines of anticipation and obviousness. In the alternative, as this Court's review rests on the entire record before the USPTO, including the findings of the Examiner, the latter's findings that the recited prior art does not disclose the

preamble limitation of "a sequence of processing steps" would support reversal of the PTAB's rejections of claims 1-5 as anticipated and/or obvious.

ARGUMENT

Claims 1-5 of the '994 Patent stand rejected by the PTAB as anticipated by Lee, and obvious in light of (principally) Lee and Gelles. *See supra* pp. 19–22; A22–25, 26–28. While "the Board's factual determinations underlying its rulings on anticipation and obviousness are reviewed under the substantial evidence test," *Rapoport v. Dement*, 254 F.3d 1053, 1058 (Fed. Cir. 2001), "[a] determination of anticipation or obviousness . . . necessarily hinges on the proper interpretation of a claim . . . ." *In re Gold*, No. 94-1038, 1994 WL 233115, at *2 (Fed. Cir. May 31, 1994). Here, the PTAB concedes that it made no factual determinations underlying its rulings, but rather rested upon its interpretation of claim 1. *See* A38.

The principal question on Illumina's appeal, therefore, "is whether the Board committed reversible error in concluding that the preamble[] of" the '994 Patent's claim 1 "did not serve to further limit the claim[]." *In re Gold*, 1994 WL 233115, at *2. As set forth below, the PTAB's interpretation of claim 1 is legally deficient, and its resulting rejection of claims 1-5 cannot stand.

I. The PTAB Erred in Construing the Preamble to Claim 1 of the '994 Patent.

"In reexamination, claims are to be given their broadest reasonable interpretation consistent with the specification, and claim language should be read

in light of the specification as it would be interpreted by one of ordinary skill in the art." *In re NTP, Inc.*, 654 F.3d 1279, 1287 (Fed. Cir. 2011) (quotation and alteration omitted). In other words, "[d]uring the patent examination process, claims receive their broadest reasonable meaning," but "this does not relieve the PTO of its ***essential task*** of examining the entire patent disclosure to discern the meaning of claim words and phrases." *Rowe*, 112 F.3d at 480 (emphasis added). Indeed, absent consistency with the entire patent disclosure, an interpretation of claim language could hardly be "reasonable." *See, e.g., In re Rehrig Pacific Co.*, 461 F. App'x 942, 946 (Fed. Cir. 2012) (concluding that Board's broad interpretation of claim was not "reasonable" in light of the specification).

This Court reviews the PTAB's claim construction *de novo* for such reasonableness, *see*, *e.g.*, *In re Morris*, 127 F.3d 1048, 1055 (Fed. Cir. 1997), a standard that the PTAB's construction of claim 1's preamble fails to satisfy.

A. Construction of a Claim's Preamble Requires a Fact-Intensive Review of the Patent Record As a Whole.

Contrary to the impression given by the PTAB's recitation of the law, *see* A23, "it is not unusual for this court to treat preamble language as limiting . . .," *Bicon, Inc. v. The Straumann Co.*, 441 F.3d 945, 952 (Fed. Cir. 2006).[15] Still,

[15] *See also*, *e.g.*, *In re Jasinski*, 508 F. App'x 950 (Fed. Cir. 2013); *C.W. Zumbiel Co. v. Kappos*, 702 F.3d 1371 (Fed. Cir. 2012); *In re Rehrig Pacific Co.*, 461 F. App'x 942 (Fed. Cir. 2012); *In re Dash*, 118 F. App'x 488 (Fed. Cir. 2004); *Griffin v. Bertina*, 285 F.3d 1029 (Fed. Cir. 2002); *Rapoport v. Dement*, 254 F.3d

"[n]o litmus test can be given with respect to when the introductory words of a claim, the preamble, constitute a statement of purpose for a device or are, in themselves, additional structural limitations of a claim." *In re Gold*, 1994 WL 233115, at *3.[16] As the PTAB recited, *see* A23, "the somewhat circular, and oft-repeated rule is that language in the preamble further limits the claim if such is necessary to give meaning to the claim[s] and properly define the invention." *In re Gold*, 1994 WL 233115, at *3 (quotations omitted) (citing cases).

Given the circularity of the verbal formulation, the crux of construing preamble language is an interpretive process: "Whether preamble language is 'necessary' or 'essential' is 'a matter to be determined on the facts of each case in view of the claimed invention ***as a whole***.'" *Id*. (quoting *In re Stencel*, 828 F.2d

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1053 (Fed. Cir. 2001); *Rowe v. Dror*, 112 F.3d 473 (Fed. Cir. 1997); *In re Paulsen*, 30 F.3d 1475 (Fed. Cir. 1994); *In re Gold*, No. 94-1038, 1994 WL 233115 (Fed. Cir. May 31, 1994); *MEMs Tech. Berhad v. ITC*, 447 F. App'x 142 (Fed. Cir. 2011); *Bicon, Inc. v. The Straumann Co.*, 441 F.3d 945 (Fed. Cir. 2006).

[16] In some circumstances, moreover, a statement of purpose itself may provide a limitation. For example, where "it would not have been obvious" to combine alleged prior art references "unless one had in mind the purpose taught by [the patent]" and "set forth in the claims themselves," the preamble "is more than a mere statement of purpose; and that language is essential to particularly point out the invention defined by the claims." *In re Stencel*, 828 F.2d 751, 755 (Fed. Cir. 1987) (quotation omitted) (finding applicant "not inhibited from claiming his [invention], limited by the statement of its purpose, and further defined by the remaining clauses of the claims at issue, when there is no suggestion in the prior art of [the invention] having the claimed structure and purpose."). Here, the preamble similarly defines the invention defined by the '994 Patent and the function for which it must be suitable. *See infra*.

751, 754 (Fed. Cir. 1987)) (emphasis added). In other words, "[t]he inquiry involves examination of the ***entire patent record*** to determine what invention the patentee ***intended*** to define and protect." *Rowe*, 112 F.3d at 478 (emphases added). *See also*, *e.g.*, *Bicon, Inc.*, 441 F.3d at 952 ("[W]hether to treat a preamble as a claim limitation is determined on the facts of each case in light of the claim as a whole and the invention described in the patent." (quotation omitted)).

The PTAB, however, did not address the requisite full scope of the '994 Patent record in its sparse, three-paragraph discussion of the preamble (largely devoid of citation). *See* A24–25.[17] Upon review of the entire '994 Patent record—the claim, specification, and prosecution history—the preamble to claim 1 defines a fundamental element of the intended invention and the specific object for which the invented system must be suitable. *See infra*. Because the preamble thus recites more than "mere introductory language," *In re Paulsen*, 30 F.3d 1475, 1479 (Fed. Cir. 1994), it limits claim 1, and the PTAB's construction to the contrary is unreasonable. *See In re Gold*, 1994 WL 233115, at *1 ("Because the Board improperly failed to accord patentable significance to limitations found in the

[17] The paucity of reasoning in a PTAB decision may itself present grounds for vacating a decision. *See In re Hruby*, 373 F.2d 997, 1001 (C.C.P.A. 1967) ("We find in the opinion, however, no citation of authority . . ., no indication that the terms mean anything other than what they say . . . other than the board majority's inability to conceive that it could be . . . ."); *Gechter v. Davidson*, 116 F.3d 1454, 1457 (Fed. Cir. 1997) (vacating Board decision because Board did not make adequate fact findings to allow judicial review or to understand the reasoning of the Board).

preambles of the rejected claims, we *reverse*." (emphasis original)); *Rowe v. Dror*, 112 F.3d 473 (Fed. Cir. 1997).

B. The Preamble Language Provides Antecedent Basis to a Key Element in the Succeeding Claim Limitations.

The starting point for interpreting the preamble is the language of the entire claim. In full, claim 1 of the '994 Patent claims:

> A system ***for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps***, the system comprising:
>
> a planar array of uniformly sized spherical microparticles, wherein the coefficient of variation of the diameters of said microparticles is less than five percent;
>
> an optical train effective to collect and focus ***the sequence of optical signals*** from the microparticles, and to record at least one optical characteristic of each microparticle which can be used to determine the approximate center of said microparticle;
>
> an imaging device onto which ***said signals*** are focused, effective to generate and record a sequence of digital images of the microparticles, with sufficient resolution for individual microparticles to be distinguished; and
>
> signal tracking means effective to correlate ***the optical signals*** from each of the microparticles in each of the sequence of digital images with said center of said microparticle.

'994 Patent, claim 1 (A69) (emphases added). Among other things, *see infra*, the preamble phrase thus defines the object to which the claimed system must be suitable.

In the PTAB's view, the limitations of "an optical train," "an imaging device," and a "signal tracking means" include functions and structures that limit the claim, whereas the preamble "does not further limit or define the . . . recited structures." A24. The PTAB's decision, however, ignores the overall structure of the claim.

"A claim's preamble may limit the claim when the claim drafter uses the preamble to define the subject matter of the claim." *Zumbiel Co.*, 702 F.3d at 1385 (quotation omitted). For example, the preamble language is limiting "when the claim(s) depend on it for antecedent basis . . . ." *Id*. *See also Bicon, Inc.*, 441 F.3d at 952 (quotation omitted).

Here, the three limitations—"an optical train," "an imaging device," and a "signal tracking means"—considered by the PTAB to limit the claims, *see* A24, all refer to "the sequence of optical signals" recited in the preamble. *See* '994 Patent, claim 1 (A69) (referring to "the sequence of optical signals," *id*., 27:51–52, "said signals," *id*., 27:56, and "the optical signals," *id*., 28:46–47). That "sequence of optical signals," however, is **defined** in the preamble as arising and being collected "during a sequence of processing steps." *Id*. (A69).

While the PTAB dismissed the preamble as defining only "an environment in which the system is capable of being used," A25, the preamble also defines the source of "the sequence of optical signals," which are produced ***by*** the "sequence

of processing steps," recited throughout the claim. This is confirmed by the specification. *See infra* pp. 35–40.

Absent the preamble, the subsequent limitation description of a "sequence of optical signals" is little more than "empty language." *Griffin*, 285 F.3d at 1033 ("Consideration of the preamble gives meaning and purpose to the manipulative steps in this case."). Otherwise, what is the sequence of optical signals, where does it emanate, and how? "The preamble is thus a necessary limitation," *see id*., and the PTAB erred in its conclusory assertion that the preamble does not "limit or define" claim 1. *See* A24–25. *Compare Zumbiel Co.*, 702 F.3d at 1385 (finding that preamble phrase "plurality of containers" gave antecedent basis to subsequent use of "containers"). *See also Jansen v. Rexall Sundown, Inc.*, 342 F.3d 1329, 1333 (Fed. Cir. 2003) (noting "it is natural to interpret the nearly parallel language . . . in the same way"); *Pitney Bowes, Inc. v. Hewlett-Packard Co.*, 182 F.3d 1298, 1305 (Fed. Cir. 1999) (preamble limits where "that statement is intimately meshed with the ensuing language in the claim").

The Court's discussion of a claim preamble in *Rapoport v. Dement*, 254 F.3d 1053 (Fed. Cir. 2001), is instructive. The claim in that case involved "[a] method for treatment of sleep apneas comprising administration of" certain compounds "to a patient in need of such treatment." *Id*. at 1056. Although the parties agreed that the preamble phrase "treatment of sleep apneas" should be treated as a claim

limitation, the Court confirmed that agreement because, "without treating the phrase 'treatment of sleep apneas' as a claim limitation, the phrase 'to a patient in need of such treatment' would not have a proper antecedent basis." *Id*. at 1059. In other words, just because the claim's body only repeated the word "treatment" from the preamble, the Court held that the full phrase "treatment of sleep apneas" limited the claim, as "of sleep apneas" in the preamble defined the "treatment" subsequently referred to in the claim. Likewise, here, the preamble phrase "during a sequence of processing steps" must be included in the limitation of the claims because that phrase defines the "sequence of optical signals" subsequently referred to in the claim.

Although the Examiner expressly (and correctly) found that the preamble language provides "antecedent basis" for the claim, *see* A195, the PTAB addressed neither the Examiner's finding nor the relevant legal principles, *see* A24–25. The PTAB ultimately relied on conclusory labeling of the preamble as a "method" that could not be read into a "system." *See* A40. In addition to offering no legal support for this conclusion, the process required for interpreting a preamble's weight, *see supra* pp. 27–28, demonstrates that labels (*e.g.*., "method" versus "system") are not determinative. Rather, the same claim language may possess attributes of different types of claims. *See*, *e.g.*, *Gemtron Corp. v. Saint-Gobain Corp.*, 572 F.3d 1371, 1378 (Fed. Cir. 2009) (noting claim language could support

functional or structural reading); *Perkin-Elmer Corp. v. Computervision Corp.*, 732 F.2d 888, 896 (Fed. Cir. 1984) (concluding that preamble to system claim was limiting).

Thus, instead of labels, "context" identifies the meaning of claim language. *See, e.g.*, *Regents of the Univ. of Minnesota v. AGA Med. Corp.*, 717 F.3d 929, 938 (Fed. Cir. 2013) (quotation omitted). Indeed, an analogous formalistic argument was rejected in *Miken Composites, LLC v. Wilson Sporting Goods Co.*, 515 F.3d 1331 (Fed. Cir. 2008), where the patentee challenged the district court's claim construction as "impermissibly import[ing] a process limitation into a product claim" in construing the term "insert." *Id.* at 1337–38. The Court, however, rejected such formalism, noting that the "claims and written description" provided the construction for "insert." *See id.* at 1337. As is true in any construction of preamble language, *see supra* pp. 27–28, the key inquiry is the meaning ascribed by the claim, *see supra*, and specification, *see infra* pp. 35–42.

That, as here, the disputed language "has functional attributes does not change the fact that the claim recites a structural component, albeit one possessed with certain understood characteristics." *Miken*, 515 F.3d at 1337–38 (noting, for example that structural elements can be expressed in functional terms). Here, the "understood characteristics" of the preamble's "sequence of processing steps," are found, not in the dictionary as in *Miken*, but in the '994 specification, *see infra* pp.

35–42; *see also* A195–96, consistent with the requirement that preamble language be viewed in light of the entire patent record.[18]

C. The '994 Specification Confirms That Claim 1's Preamble is Entitled to Patentable Weight.

Unlike the analysis performed by the PTAB, *see* A24–25, "[t]he court looks next to the specification and drawings to determine whether those sources convey a clear structural meaning" for the disputed preamble phrase. *Rowe*, 112 F.3d at 479. *See also id*. at 479–80 (relying on specification and drawings). In this case, the written description of the '994 Patent (1) discloses that the "sequence of optical signals" at the center of claim 1's limitations is "intimately meshed," *Pitney Bowes*, 182 F.3d at 1306, with the preamble's statement that those signals emerge from and are studied "during a sequence of processing steps," and (2) describes the associated processing structure and "component[s]" that the PTAB believed wanting, *see* A25.

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[18] The PTAB's parallel conclusion that the preamble language cannot be limiting because the elements of the processing steps "are not recited in the claim," A40; *see also* A25, likewise ignores the specification, which the Examiner found to give meaning to the preamble phrase, *see, e.g.*, A195–96; A288. By the same token, the PTAB's reliance on the absence of these elements—by which the specification defines processing steps, *see infra*—is at odds with the PTO's obligation to afford the broadest reasonable interpretation because it ignores the "[o]pen claim language" represented by use of the word "comprising." *Magsil Corp. v. Hitachi Global Storage Techs., Inc.*, 687 F.3d 1377, 1383 (Fed. Cir. 2012). Contrary to the PTAB's stilted construction, "[t]he transition 'comprising' creates a presumption that the recited elements are only a part of the [invention], that the claim does not exclude additional, unrecited elements." *Id*. (quotation omitted).

1. The Preamble is Limiting Because It Describes the Essence of the Claimed Invention as Set Forth in the Specification.

Both the intimate connection between the "sequence of optical signals" and the "sequence of processing steps," and the components of the processing steps begin in the '994 Abstract's description of the invention. In that initial introduction to the invention, the '994 Patent describes the attachment of analytes to microparticles thereafter disposed "inside of a flow chamber where ***steps of an analytical process*** are carried out by delivering a ***sequence of processing reagents*** to the microparticles ***by a fluidic system*** under microprocessor control." '994 Patent, Abstract (A44) (emphases added).[19] Indeed, the abstract makes clear that the preamble "processing steps" are the source—and not simply the environment—of the sequence of optical signals, explaining that, "[***i***]***n response to such process steps, an optical signal is generated*** at the surface of each microparticle . . . ." *Id.* (A44) (emphasis added). *See also id*., 1:66 through 2:19 (A56).

The '994 specification next defines the problems solved by the invention in "handling and manipulating large numbers of microparticles, e.g. tens to hundreds of thousands . . . ." *Id*., 1:40–43 (A56). The problems the inventors set out to resolve include "how to track individual microparticles ***through multiple steps of a***

[19] The Abstract likewise expressly ties the "sequence of processing steps" to the "signal tracking" limitation relied upon by the PTAB. *See* A44 ("A key feature of the invention is the correlation of the sequence of optical signals generated by each microparticle in the planar array during the analytical process.").

process, . . . the ability to uniformly deliver reagents to microparticles for ***carrying out steps*** of an analytical process," and various "disruption[s]" relating to delivery of processing reagents to the microparticles—and the analytes attached thereto—being studied. *Id*., 1:43–56 (A56) (emphases added).

This Court recognized the significance of a patent's definition of such goals in *In re Gold*, No. 94-1038, 1994 WL 233115 (Fed. Cir. May 31, 1994). There, as here, the Board determined that the preamble language "does not set forth or require any specific structure" and simply describes "the environment for using the apparatus." *Id*. at *3. Again, as in this case, however, "[t]he specification of the patent . . . makes clear that [the applicant] was working on [a] particular problem . . .," there, human underwater signaling, *see id*., and here the difficulties in subjecting hundreds of thousands of microparticles to, and tracking them through, the steps of an analytical process. *See supra*. To construe the preamble to ignore the patent's statement of the problem "would be divorced from reality" because such "limitations appearing in the preamble are essential to point out the invention as defined by the claims." *In re Gold*, 1994 WL 233115, at *3 (quotation omitted).

Moreover, in response to the problems defined by the '994 specification, the invention provides, among other things, "an apparatus for detecting ***optical signals generated by, or as the result of***, interactions of ***processing*** reagents and analytes," '994 Patent, 2:5–8 (A56) (emphases added), that "permit[s] the tracking and

analysis of multiple analytes anchored to separate microparticles through a sequence of several processing and/or analysis steps," *id*., 1:60–64 (A56). Again, the specification confirms that a "sequence of processing steps" produces the "sequence of optical signals" underlying claim 1.[20] Taken together, the '994 specification's identification of the problem to be resolved, and the resolution invented—both of which connect to the preamble—demonstrate "what the inventors actually invented and intended to encompass by" claim 1. *See Rowe*, 112 F.3d at 478 (describing conditions under which preamble recitations are structural limitations).[21]

The specification likewise belies the PTAB's conclusion that the preamble cannot provide a limitation because claim 1 does not expressly disclose the elements or components of the preamble "process[]." *See* A25; A40. Rather, the '994 specification supplies the requisite structure and elements for the invention:

> [A]n apparatus comprising a flow chamber for disposing a population of microparticles in a planar array; fluidic means for sequentially delivering processing reagents from one or more reagent reservoirs to

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[20] The invention's various aspects incorporate the apparatus to detect "[o]ptical signals generated by, or produced as a result of, the interaction of processing reagents and [analytes] on the microparticles . . . ." '994 Patent, 2:48–51 (A56). *See also id*., 8:48–50 (A59) ("An important feature of detection means (114) of the invention is the ability to keep track of individual microparticles through multiple process steps and/or cycles."). *See also supra* p. 11.

[21] Indeed, the PTAB conceded that "the '994 Patent teaches that the claimed system was designed to handle microparticles through a series of processing steps and collect optical signals from microparticles during these steps." A39.

> the flow chamber; and detection means for detecting a sequence of optical signals from each of the microparticles of the population.

'994 Patent, 2:20–26 (A56). The structure is further detailed in "a schematic representation" of an apparatus with such flow chamber and detection system, *see id*. at Figure 1*a* (A46); *id*., 2:60–63 (A56),[22] and schematics and illustrations of a "flow chamber," *id*., 2:64 through 3:6 (A56–57); *see also id*., Figures 1*b*, 2*a* through 2*c*, 3*a* through 3*d*, 4 (A47–51); and a "fluidics system," *id*., 3:7–8 (A57); *see also id*., Figure 5 (A52), connected to the flow chamber for conducting a series of processing steps. Indeed, "[a] key feature of ***the invention*** is a flow chamber (100)," *id*., 5:23 (A58) (emphasis added), which "is operationally associated with fluidic system (112) and detection system (114) . . .," *id*., 5:4–5:6 (A58). *See also id*., 8:4–7 (A59) ("Preferably, process reagents are delivered to flow chamber (100) by the fluidic system illustrated in [Figure 5] which has the capacity to handle many different reagents for complex analytical processes.").

As the Examiner recognized, *see* A195–196 (citing '994 Patent, Figure 8), the '994 Patent further associates a flow chamber and fluidics system within "a flow chart summarizing operation of the system of the invention," '994 Patent, 3:15–16 (A57), through a series of "process steps," *id*., 9:59 (A60); *see also id*.,

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[22] The schematic embodiment includes the structure of a flow chamber that "holds microparticles in a planar array from which optical signals (108) generated by analytes and/or reactants on microparticles can be collected and imaged," all under the control of a computer. *See id*., 4:65 through 5:6 (A57–58).

Figure 8, step 804 (A55) ("Execute Proc. Step(s)"), in which optical signals are collected from microparticles, *id*., 10:28 (A60). *See also id*., 9:32–33 (A60) (describing Figure 8 as a summation of "[t]he general operation of the system of the preferred embodiment"). Indeed, based on these disclosures, the Examiner found that the phrase " 'processing steps' has a particular meaning, namely, steps associated with the processing of microparticles in a fluidic system (e.g., exposing the microparticles to various reagents . . .)" that defines "a particular part of the operation of the system distinct from other steps." A195–196 (citing '994 Patent, Figure 8, step 804 and 8:4–40, 9:61–65).

Likewise, the preamble's requirement that the optical signals are obtained when certain other actions are also being performed implicates control circuitry, a structure of the system illustrated in the specification. *See*, *e.g.*, '994 Patent, 19:58 through 20:25 (A65) (explaining that the detection system (114) is controlled by a Sun Microsystems controller in that exemplary embodiment, and subsequently describing that images (e.g., steps 5, 8, 10) are taken during processing steps (e.g., steps 2, 9)); *id*., 9:59 through 10:5 (A60) (explaining that a computer (116) controls various components of the system to initiate a series of process steps (804), and controls the timing of the taking of the images (810) such that they occur during the series of process steps (804), (824), and (826)). The preamble language "during a series of process steps" thus, contrary to the PTAB's

assumption that the preamble only defines the environment in which the system operates, implicates concrete structures of the system.

Viewed as a whole, the '994 specification thus describes the elements and structure of an invention to subject analytes anchored to microparticles to an analytical process, and analyze the optical signals generated by the steps in that process. In fact, the specification directly exposes the error in the PTAB's specific conclusion that claim 1 lacks components such as a "fluidics controller." *Compare supra with* A25. The invention therefore accords with claim 1's preamble provision that the system be suitable for "detecting a sequence of optical signals . . . during a sequence of processing steps." '994 Patent, claim 1 (A69).

That the elements or structure of the process "are not [expressly] recited in the claim," A40, is—contrary to the PTAB's view—of no moment in assessing whether the preamble is limiting. To the contrary, a preamble properly limits a claim where, as here, the preamble refers to the "essence of the invention," but, "standing alone, the bod[y] of [the] claim[] do[es] not require" that essence. *See MEMS Tech. Berhad*, 447 F. App'x at 154. *See also Griffin*, 285 F.3d at 1033 (finding Board did not err in finding preamble limiting where preamble terms relay "the essence of this invention"); *In re Jasinski*, 508 F. App'x at 952 (Board erred in "failing to give 'patentable weight' to . . . preamble language" that referred to the "essence of the invention").

2. The Gelles Declaration Confirms that the Specification Defines the Elements of the "Sequence of Processing Steps" Recited in Claim 1's Preamble.

This Court has made clear that "claim language should be read in light of the specification as it would be interpreted by one of ordinary skill in the art." *In re NTP, Inc.*, 654 F.3d at 1287 (quotation and alteration omitted). Before the Examiner, Illumina submitted the declaration of Dr. Gelles, *see supra* pp. 17–18, to confirm the understanding of a skilled artisan with respect to the invention described by the '994 Patent.[23] Dr. Gelles's description of the invention further confirms that claim 1 incorporates the elements of the "sequence of processing steps" recited in the preamble.

Among other things, Dr. Gelles explained that "[t]he meaning of the complete phrase 'during a sequence of processing steps' is made apparent in the specification" to indicate "manipulating the environment containing the microparticles, where the manipulation includes the addition of reagents and

[23] The PTAB "conclude[d] that Dr. Gelles is qualified to testify" on the technology underlying this dispute "[b]ased on his experience and education." A15. Dr. Gelles is a Professor of Biochemistry and Molecular Pharmacology at Brandeis University with research interests in, among other things, biochemistry and molecular biophysics of complex systems involved in cellular organization and gene expression. *See* Gelles Decl. ¶¶ 1–7 (A565–66). As part of this research, Dr. Gelles "use[s] several imaging technologies . . ., including differential interference contrast microscopy and multi-wavelength single molecule fluorescence microscopy." *Id*. ¶ 5 (A565). Dr. Gelles was the first author of a prior art reference cited by the Examiner and the PTAB in rejecting certain claims of the '994 Patent. *See, e.g.*, *id*. ¶ 11 (A566).

washes to a flow chamber using a fluidics controller." Gelles Decl. ¶ 13 (A566). *See also id.* ¶¶ 14–23 (A566–67) (citing '994 Patent, 5:23–42, 8:4–5, 9:59–61, 10:24–25, 9:59 through 10:27, and Figure 8). Indeed, the "process steps are initiated" by the fluidics controller described in the '994 specification. *See id.* ¶ 17 (A567) (quoting '994 Patent, 9:59–61); *see also id.* ¶ 18 (A567). Dr. Gelles's understanding is therefore consistent with the Examiner's findings as to the "particular meaning" of the phrase "processing steps," *see* A195–96 (citing '994 Patent, Figure 8, step 804 and 8:4–40, 9:61–65), and the understanding of the preamble as a limitation to claim 1.

D. The Prosecution History Further Confirms that the Preamble is Entitled to Patentable Weight.

Along with the claim itself and the '994 specification's description of the claimed invention, the prosecution history surrounding the preamble phrase "during a sequence of processing steps" further confirms that the language imparts a limitation on claim 1. As this Court has explained, "clear reliance on the preamble during prosecution to distinguish the claimed invention from the prior art transforms the preamble into a claim limitation because such reliance indicates use of the preamble to define, in part, the claimed invention." *Invitrogen Corp. v. Biocrest Mfg., L.P.*, 327 F.3d 1364, 1370 (Fed. Cir. 2003) (quotation omitted). *See also, e.g.*, *Jansen*, 342 F.3d at 1333 ("We must . . . give [the disputed language]

weight, for the patentability of the claims hinged upon their presence in the claim language.").

As recited above, during initial prosecution the Examiner rejected the application for the '994 Patent based on, *inter alia*, a finding that Resnick disclosed "a system 'comprising signal tracking means for correlating the optical signals from each particle in each of the digital images with the position of the particle.'" *See* A528, 530, 533 (quoting June 24, 2003 Office Action (emphasis omitted)). *See supra* pp. 13–15.

At the time of rejection, the preamble of claim 1 read:

> A detection apparatus for detecting a sequence of optical signals from each of a plurality of microparticles comprising . . . .

A529. "In response," A528, to the rejection of claim 1, the applicant amended the preamble to claim 1 (deletions indicated by strikethrough and insertions indicated by underline):

> A ~~detection apparatus~~ <u>system</u> for detecting a sequence of optical signals from each of a plurality of microparticles <u>during a sequence of processing steps, the system</u> comprising . . . .

A529. The applicant then argued that the system of the invention as amended "allows the array of microparticles to be tracked during a sequence of processing steps, as described above." A533.[24] In contrast, the applicant argued, Resnick

[24] While the applicant's "sequence" argument also referred to the "imaging device" and "signal tracking means" limitations of claim 1, the only amendment to

involves study of a sample that is "fixed" prior to analysis. *See* A533 (citing U.S. Patent No. 4,125,828 at 7:1–10).

In response to this amendment and argument, the Examiner withdrew the rejection of claim 1, and the '994 Patent issued with its original four claims. The applicant's "use of" the phrase "during a sequence of processing steps," "a feature found only in the preamble . . ., to distinguish prior art supports construing the preamble as a limitation . . . ." *In re Dash*, 118 F. App'x 488, 491 (Fed. Cir. 2004) (upholding Board's consideration of preamble in construction where applicant "distinguished prior art reference" on basis found in preamble).

* * *

Considering, as this Court requires, the entire record of the '994 Patent thus makes clear that the preamble provides a limitation to claim 1 by, among other things, establishing the antecedent basis to a fundamental element recited throughout claim 1, describing the essence of the invention claimed in the '994 Patent (and claim 1 in particular), and serving as the foundation for the initial issuance of claim 1 over the Examiner's rejection in prosecution. Under the weight of this record, the PTAB's conclusory determination to the contrary is unreasonable.

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claim 1 involving such "sequence" was the addition of the "sequence of processing steps" in the preamble. *See* A529, 533. Moreover, the applicant concluded the discussion of the "imaging device" and "signal tracking means" by referring to the preamble's "sequence of processing steps." *See* A533.

II. The PTAB Erred in Entering New Rejections of Claims 1-5 of the '994 Patent Based on the PTAB's Erroneous Construction of Claim 1's Preamble.

The PTAB entered new rejections of claims 1-4 as both anticipated by Lee and obvious in light of Gelles, and claim 5 as obvious in light of, principally, Lee and Gelles. *See* A33; *see also* A28 (relying on Lee and Gelles in rejecting claim 5). Because the PTAB's rejections "were based upon" its interpretation of claim 1's preamble as non-limiting, *see, e.g.*, A38, and that interpretation is erroneous in light of the '994 Patent's entire record, *see supra*, the PTAB's resulting rejections cannot stand. *See, e.g.*, *In re Gold*, 1994 WL 233115, at *2 ("A determination of anticipation or obviousness . . . necessarily hinges on the proper interpretation of a claim . . . .") (reversing Board's finding of anticipation where Board erred in failing to read preamble as limiting and erred in finding preamble, if limiting, was anticipated or obvious over prior art).[25]

Although Life Tech alternately argued to the PTAB that, among other references, Lee and/or Gelles disclose a "sequence of processing steps" as recited in the preamble and specification, *see* A354–58, the PTAB did not address this argument or substantively consider whether Lee or Gelles disclose the preamble limitation. Because anticipation, *see, e.g.*, *In re Cederblad*, 4 F. App'x 914, 916

[25] Indeed, without its claim construction, the PTAB's determinations of anticipation and obviousness are "not based on any evidence in the record" considered by the Board "and, therefore, lack[] substantial evidence support." *In re Zurko*, 258 F.3d 1379, 1385 (Fed. Cir. 2001).

(Fed. Cir. 2001); *In re Gleave*, 560 F.3d 1331, 1334–35 (Fed. Cir. 2009), and the differences between the claimed invention and the alleged prior art to determine obviousness, *see*, *e.g.*, *Zumbiel Co.*, 702 F.3d at 1379, are questions of fact on which the PTAB made "no [factual] findings," at the very least this Court must vacate the existing rejections and "remand for further proceedings with respect to those claims." *In re Cederblad*, 4 F. App'x at 918. *See also In re Sang-Su Lee*, 277 F.3d 1338 (Fed. Cir. 2002) (vacating and remanding decision in order for Board to make factual findings).

As an alternative to remand, the Examiner's findings with respect to the prior art, *see*, *e.g.*, *supra* pp. 16–17; *infra*, fully demonstrate that Lee and Gelles do not disclose the preamble limitation of "detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps." '994 Patent, claim 1 (A69). Indeed, this Court conducts its review "on the record before the Patent and Trademark Office." 35 U.S.C. § 144. *See In re Ditto*, 499 F. App'x 1, 3 (Fed. Cir. 2012) ("[T]he Board's factual determinations, ***including what the examiner considered*** during prosecution, are reviewed for substantial evidence." (quotation omitted) (emphasis added)); *In re Pennington Seed, Inc.*, 466 F.3d 1053, 1058 (Fed. Cir. 2006) (finding substantial evidence supported examiner's determination in trademark case).

On that overall record, the Examiner's findings are supported by substantial evidence, *see In re Avid Identification Sys., Inc.*, 504 F. App'x 885, 888 (Fed. Cir. 2013) ("A finding is supported by substantial evidence if a reasonable mind might accept the evidence to support the finding."); *In re Pond*, 466 F. App'x 876, 879 (Fed. Cir. 2012) ("Substantial evidence is something less than the weight of the evidence but more than a mere scintilla of evidence . . . ." (quotation omitted)), and thus support reversal of the PTAB's rejections of claims 1-5 as anticipated and/or obvious.

Indeed, after (correctly) affording patentable weight to claim 1's preamble, the Examiner examined the Lee and Gelles references underlying the PTAB's subsequent rejections, and found that the references do not subject microparticles "to processing steps as defined by the ['994 Patent] specification during the observation of the microparticles." A202. *See also* A211. Rather, the Examiner found that (1) "Lee generally discloses that the microparticles are prepared, attached to cells and a membrane, ***then*** observed," A202 (citing Lee, 9:22 through 10:14 (A498)) (emphasis added); and (2) "Gelles generally discloses that the microparticles are prepared with kinesin, applied to microtubules adhering to a glass coverslip, ***then observed***," A211 (emphasis added); *see also* Gelles (A517–20). Because, as the Examiner found, Lee and Gelles do not "specifically disclose detecting a sequence of optical signals from the microparticles ***during*** a sequence

of processing steps," *see* A202, 211 (emphasis added), the PTAB's rejections of claims 1-5 should be reversed.

CONCLUSION

The Court should vacate the PTAB's rejections of claims 1-5 of the '994 Patent and remand claims 1-5 to the Board to make factual findings regarding patentability, or, in the alternative, reverse the PTAB's rejections in light of the substantial evidence relied upon by the Examiner in finding claims 1-5 not anticipated or obvious.

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Dated: June 23, 2014

ADDENDUM

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
95/000,529	02/19/2010	6831994	048522-0003	8420

22922 7590 02/26/2013

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ART UNIT	PAPER NUMBER
3992	

MAIL DATE	DELIVERY MODE
02/26/2013	PAPER

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Illumina, Inc.
(Patent Owner and Appellant)

v.

Life Technologies Corporation
(Requester and Cross-Appellant)

Appeal 2012-010385
Reexamination Control No. 95/000,529
US Patent 6,831,994 B2
Technology Center 3900

Before RICHARD M. LEBOVITZ, JEFFREY B. ROBERTSON, and RAE LYNN P. GUEST, *Administrative Patent Judges*.

LEBOVITZ, *Administrative Patent Judge*.

DECISION ON APPEAL

This is a decision on appeal in an inter partes reexamination of U.S. 6,831,994 B2. The Patent Owner appeals the Examiner's decision to reject claims as anticipated and obvious. The Third Party Requester cross-appeals the Examiner's determination not to adopt rejections of claims as obvious and the Examiner's withdrawal of the rejection for impermissibly broadening the scope of the claims. The Board's jurisdiction for this appeal is under 35 U.S.C. §§ 6(b), 134, and 315. We reverse and enter new grounds of rejection.

STATEMENT OF THE CASE

The patent in dispute in this appeal is U.S. Patent No. 6,831,994 B2, issued December 14, 2004 (hereinafter, "the '994 patent"). The Patent Owner and Real Party in Interest is Illumina, Inc. ("Illumina"). Illumina's Appeal Brief 2, September 13, 2011 ("Illumina Appeal Br.").

A replacement request for inter partes reexamination of the '994 Patent was filed on February 19, 2010 by a Third-Party Requester under 35 U.S.C. §§ 311-318 and 37 C.F.R. §§ 1.902-1.997. Request for Inter Partes Reexamination 1. The Third-Party Requester is Life Technologies Corporation ("Life"). Life Respondent Brief 1, October 12, 2011 ("Life Resp't Br.").

The '994 patent was the subject of litigation in *Life Technologies Corp. v. Illumina, Inc.*, No.1:09-cv-00706-RK (D. Del., filed September 21, 2009). The Delaware Court issued a claim construction order on December 15, 2010. On April 6, 2011, the Delaware District Court ordered the transfer

of the litigation to the Southern District of California, where the case is designated 3:11-cv-00703-JAH-POR. Illumina Appeal Br. 2.

Related U.S. Patent 6,654,505 was subject of inter partes reexamination, Application 95/001,292. An appeal was decided in that case. *Decision on Appeal* in Appeal No. 2012-007309, mailed November 29, 2012.

In the present reexamination appeal, an oral hearing took place on December 13, 2012. A transcript of the oral hearing was entered into the record on January 24, 2013.

Claims

Claims 1-35 are pending. Claims 1-24, 26-30, and 33-34 stand rejected by the Examiner. Claims 25, 31, and 35 are allowed by the Examiner. Claim 32 is canceled. The rejections by the Examiner of claims 1-24, 26-30, and 33-34 are appealed by Illumina. Illumina App. Br. 5. Life cross-appeals the Examiner's determination not to adopt proposed rejections of the claims. Life Technologies Appeal Brief 5, filed October 13, 2011 ("Life Appeal Br.").

Claim 1 reads as follows (bracketed numerals added for reference to the main limitations; underlining indicates amendments relative to the original claims):

> 1. A system for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps, the system comprising:
>
> [1] a planar array of uniformly sized spherical microparticles, wherein the coefficient of variation of the diameters of said microparticles is less than five percent;
>
> [2] an optical train effective to collect and focus the sequence of optical signals from the microparticles, and to

record at least one optical characteristic of each microparticle which can be used to determine the approximate center of said microparticle;

[3] an imaging device onto which said signals are focused, effective to generate and record a sequence of digital images of the microparticles, with sufficient resolution for individual microparticles to be distinguished; and

[4] signal tracking means effective to correlate the optical signals from each of the microparticles in each of the sequence of digital images with said center of said microparticle.

7. (Amended) The system of claim 1, wherein the system is configured to designate a first pixel for determining characteristics of an optical signal generated at a microparticle, the first pixel being correlated with the center of the microparticle.

APPEAL BY ILUMINA

Illumina appeals the following rejections by the Examiner (Illumina Appeal Br. 5):

1. Rejection of claims 1-4, 6, 14-24, 26-30, and 33-34 under 35 U.S.C. § 102 as anticipated by Brenner;[1]

2. Rejection of claims 7-10 and 13 under 35 U.S.C. § 103 as obvious over Brenner in view of Schmidt;[2]

3. Rejection of claims 7-10 and 13 under 35 U.S.C. § 103 as obvious over Brenner in view of Gelles;[3]

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[1] Sydney Brenner, *Molecular Tagging System,* WO 96/12014 (published April 25, 1996) (Brenner).

[2] Christine E. Schmidt et al., *Integrin-Cytoskeletal Interactions in Migrating Fibroblasts are Dynamic, Asymmetric, and Regulated,* J. Cell Biology, 123(4):977-991, 1993 (Schmidt).

4. Rejection of claims 7-12 under 35 U.S.C. § 103 as obvious over Brenner in view of Hicks;[4] and

5. Rejection of claim 5 under 35 U.S.C. § 103 as obvious over Brenner in view of Hansen.[5]

CLAIM INTERPRETATION

Limitation [4] of claim is in dispute in this appeal. We thus begin by construing limitation [4] in view of the '994 patent specification.

The term "signal tracking means" is a means-plus-function term that invokes 35 U.S.C. §112 ¶ 6. Its scope defined by the structure disclosed in the specification plus any equivalents of that structure. *See In re Donaldson*, 16 F.3d 1189, 1195 (Fed. Cir. 1994) (en banc).

The '994 patent specification does not use the term "signal tracking means." However, it does describe the function of the signal tracking means recited in the claim ("to correlate the optical signals from each of the microparticles in each of the sequence of digital images with said center of said microparticle") and a structure which accomplishes the recited function.

As explained in the '994 patent specification and reflected in the claims, optical signals are collected from the microparticles and used to

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[3] Jeff Gelles et al., *Tracking kinesin-driven movements with nanometer-scale precision,* Nature, 331:450-453, 1988 (Gelles).
[4] B.W. Hicks et al., *Tracking Movements of Lipids and Thy1 Molecules in the Plasmalemma of Living Fibroblasts by Fluorescent Video Microscopy with Nanometer Scale Precision,* J. Membrane Biology, 144(3):231-244, 1995 (Hicks).
[5] W. Peter Hansen et al., *Light Scatter-Based Immunoassay Without Particle Self Aggregation*, U.S. Pat 5,589,401 (granted December 31, 1996) (Hansen).

generate a sequence of digital images. This function is performed by the imaging device [3] of claim 1. Once the optical signals are recorded in successive digital images, the [4] signal tracking means performs the function of correlating the optical signals from each of the microparticles in each of the sequence of digital images with the center of the microparticle.

Findings of Fact

[FF1][6]

The '994 patent specification describes a "detection means" with an "important feature" of having the "ability to keep **track** of individual microparticles through multiple process steps and/or cycles." Col. 8, ll. 48-50 (emphasis added).

[FF2]

The detection means (114) is shown in Figure 1a of the patent as comprising a microscope, CCD (charge-coupled device which is capable of generating a digital image), and computer. See col. 5, ll. 7-12; col. 8, ll. 48-54.

[FF3]

In connection with the tracking, the '994 patent explains that the "detection means (114) periodically records optical characteristics of individual microparticles that provide a close approximation microparticle centers." Col. 8, ll. 51-54. This function appears to correspond to the imaging device [3] of claim 1, such as the CDD device shown in Figure 1A (FF2).

[6] "FF" refers to a Finding of Fact.

[FF4]

Once microparticle centers are determined, the patent describes assigning pixels "for determining characteristics, e.g. intensity, of an optical signal generated at each microparticle (602)." Col. 9, ll. 6-9.

[FF5]

The patent describes a preferred embodiment in which "initial pixel is assigned which encloses the computed center of a microparticle." Col. 9, ll. 24-26.

[FF6]

The '994 patent specification explains what structure performs the pixel assignment (FF4 & FF5):

> After the fluorescent image is collected, the focal plane of the microscope objective is returned (814) to the microparticle focal plane, where another image is collected (816) for the purpose of computing microparticle centers as described above. **The image of microparticle centers is transferred to data server (812) where data processor (818) assigns pixels of the fluorescent image to each microparticle center, as described above**.

Col. 10, ll. 13-20 (emphasis added).

Analysis

The claimed correlation of the "the optical signals from each of the microparticles in each of the sequence of digital images with said center of said microparticle" is thus performed by the "data processor." As the "data processor" performs the computational function of assigning pixels of the fluorescent images to the microparticle centers, it would be understood by one of ordinary skill in the art to be a component of the computer shown in Figure 1a (FF2).

> In cases involving a computer-implemented invention in which the inventor has invoked means-plus-function claiming, [the Federal Circuit] has consistently required that the structure disclosed in the specification be more than simply a general purpose computer or microprocessor.
>
> That was the point made by this court in *WMS Gaming, Inc. v. International Game Technology*, 184 F.3d 1339 (Fed. Cir. 1999). In that case, the court criticized the district court, which had determined that the structure disclosed in the specification to perform the claimed function was "an algorithm executed by a computer." The district court erred, this court held, "by failing to limit the claim to the algorithm disclosed in the specification." *Id.* at 1348. The rationale for that decision is equally applicable here: a general purpose computer programmed to carry out a particular algorithm creates a "new machine" because a general purpose computer "in effect becomes a special purpose computer once it is programmed to perform particular functions pursuant to instructions from program software." *Id.*, quoting *In re Alappat*, 33 F.3d 1526, 1545 (Fed. Cir. 1994). The instructions of the software program in effect "create a special purpose machine for carrying out the particular algorithm." *WMS Gaming*, 184 F.3d at 1348. Thus, in a means-plus-function claim "in which the disclosed structure is a computer, or microprocessor, programmed to carry out an algorithm, the disclosed structure is not the general purpose computer, but rather the special purpose computer programmed to perform the disclosed algorithm." *Id.* at 1349.

Aristocrat Technologies Australia Pty Ltd. v. International Game Technology, 521 F3d 1328, 1333 (Fed. Cir. 2008).

It is thus made clear by *Aristocrat* that when a computer-implemented invention is being claimed – as is the case here – the computer is not a general purpose one, but rather a computer programmed to perform the recited function, which in this case is "to correlate the optical signals from

each of the microparticles in each of the sequence of digital images with said center of said microparticle." The claimed "signal tracking mean" is therefore interpreted in light of the '994 patent specification to require a computer, or equivalent data processing structure, which comprises software or hardware that enables it to perform the claimed correlation function of limitation [4].

The claim requires [3] an "imaging device" which "record[s]" digital images and a [4] "signal tracking means" which correlates the digital images with microparticle centers. The recordation of the image is therefore explicitly recited in the claim to be carried out separately from the subsequent correlation which is accomplished with the signal tracking means. The correlation is performed in the '994 patent by a process involving the assignment of pixels to the microparticle center (FF4-FF6). However, we do not limit the claimed "correlate" limitation to this pixel assignment process.

1. ANTICIPATION BY BRENNER

Claims 1-4, 6, 14-24, 26-30, 33, and 34 stand rejected under 35 U.S.C. § 102 as anticipated by Brenner. The issue is this rejection is as follows:

Does Brenner describe [4] "signal tracking means effective to correlate the optical signals from each of the microparticles in each of the sequence of digital images with said center of said microparticle"?

Findings of Fact

[FF7]

> Typically, the sorted molecules are exposed to ligands for binding, e.g. in drug development, or are subjected chemical or

> enzymatic processes, e.g. in polynucleotide sequencing. In both of these uses it is often desirable to simultaneously observe signals corresponding to such events or processes on large numbers of microparticles.

Brenner, p. 25, ll. 34-38

[FF8]

> Preferably, whenever light-generating signals, e.g. chemiluminescent, fluorescent, or the like, are employed to detect events or processes, loaded microparticles are spread on a planar substrate, e.g. a glass slide, for examination with a scanning system . . . . The scanning system should be able to reproducibly scan the substrate and to define the positions of each microparticle in a predetermined region by way of a coordinate system. In polynucleotide sequencing applications, it is important that **the positional identification of microparticles be repeatable in successive scan steps**.

Brenner, p. 26, ll. 3-10 (emphasis added).

[FF9]

> Such scanning systems may be constructed from commercially available components, e.g. x-y translation table controlled by a digital computer used with a detection system comprising one or more photomultiplier tubes, or alternatively, a CCD array, and appropriate optics, e.g. for exciting, collecting, and sorting fluorescent signals . . . **Computer software** for table translation and data collection functions can be provided by commercially available laboratory software, such as Lab Windows, available from National Instruments.

Brenner, p. 26, 11-23 (emphasis added).

[FF10]

> **The output of the photon counters is collected by computer 304, where it can be stored, analyzed, and viewed on video 360**.

Brenner, p. 26, l. 38 to p. 27, l. 1 (emphasis added).

[FF11]

> The stability and reproducibility of the positional localization in scanning will determine, to a large extent, the resolution for separating closely spaced microparticles. Preferably, the scanning systems should be capable of resolving closely spaced microparticles, e.g. separated by a particle diameter or less.

Brenner, p. 27, ll. 4-7.

Discussion

The issue is this rejection is whether Brenner describes the claimed system comprising a [4] "signal tracking means effective to correlate the optical signals from each of the microparticles in each of the sequence of digital images with said center of said microparticle." Limitation [4] appears in both independent claims 1 and 29 involved in this appeal.

Brenner describes a computer loaded with software for determining the position of microparticles and correlating these positions in successive images (FF8 & FF11). Brenner also describes detecting optical signals from the particles (FF8 & FF11) as recited in claim 1. However, the Examiner did not provide sufficient evidence that Brenner teaches a computer that is able to correlate the optical signals with the microparticle centers as required by limitation [4] of the claims.

The Examiner states that Brenner performs a correlation with microparticle centers, citing Brenner's disclosure at page 26, lines 7-10 & 11-38; page 35, lines 23-39. RAN ("Right of Appeal Notice") 6. We have reviewed this disclosure and find evidence that Brenner registers the positions of microparticles in an array, but none that Brenner determines the microparticle centers.

Life contends that Brenner's "two-dimensional images include positional registration information for the microparticle centers" and cites page 35, lines 23-34 for support. Life Resp't Br. 8. The most pertinent passage in this disclosure is as follows:

> The avidinated slide with the attached microparticles is examined with a scanning fluorescent microscope. . . . The excitation beam and fluorescent emissions are delivered and collected respectively, through the same objective lens. . . . The computer generates a two dimensional map of the slide which registers the positions of the microparticles.

In other words, Life takes the position that because a complete scan was accomplished of the slide on which the microparticles emitting fluorescence optical signals were arrayed, Brenner would necessarily measure the optical signals of the microparticle centers. This argument is not persuasive.

The claim requires a [4] "signal tracking means . . . to correlate the optical signals from each of the microparticles in each of the sequence of digital images with said center of said microparticle." We interpreted the correlation to be accomplished separately from the recordation of the images, the latter which is carried out by the "imaging device." *See Claim interpretation*, supra at p. 8-9. Brenner's teaching that the entire slide is scanned, which might include an image of the optical signals associated with the microparticle centers, corresponds to a step accomplished by the imaging device of claim 1. However, a complete scan does not satisfy limitation [4] because limitation [4] requires a structure, such as a computer or other data processing structure, which correlates the optical signals with the microparticle centers; an image of the optical signals and microparticle

centers is insufficient because it lacks a correlation step performed by a computer.

For the foregoing reasons, we reverse the rejection of claims 1-4, 6, and 14-24, 26-30, 33, and 34 under 35 U.S.C. § 102(b) as anticipated by Brenner.

2. OBVIOUSNESS IN VIEW OF BRENNER AND SCHMIDT

Claims 7-10 and 13 stand rejected under 35 U.S.C. § 103 as obvious over Brenner in view of Schmidt.

Claim 7 is dependent on claim 1 and further recites "the system is configured to designate a first pixel for determining characteristics of an optical signal generated at a microparticle, the first pixel being correlated with the center of the microparticle.

The Examiner did not meet the burden of establishing that claim 7 would have been obvious in view of Brenner and Schmidt at the time of the invention.

Pointing to disclosure on page 979 of Schmidt, the Examiner found that Schmidt disclosed a system "configured to designate a first pixel for determining characteristics of an optical signal generated at a microparticle, the first pixel being correlated with the center of the microparticle." RAN 11. The cited disclosure from Schmidt is as follows:

"In a few cases, the centroid of the bright portion of the DIC image was determined from the weighted pixel intensity without using cross-correlation analysis." Schmidt, p. 979 (in section titled "Nanometer-precision Analysis of Bead Position").

The Examiner did not provide an explanation as to how determining the centroid of the bright portion of the DIC image from "the weighted pixel intensity" corresponds to the limitation recited in claim 7.

Illumina provided a declaration by Dr. Jeff Gelles, who was a Professor of Biochemistry and Molecular Pharmacology at Brandeis University in Waltham, Massachusetts, at the time the declaration was executed. Gelles Decl. ¶ 1. Dr. Gelles testified in his written declaration that he used "imaging technologies in [his] research, including differential interference contrast microscopy and multi-wavelength single molecule fluorescence microscopy." Gelles Decl. ¶ 5. Based on his experience and education, we conclude that Dr. Gelles is qualified to testify in this matter. Gelles Decl. ¶¶ 3-6. Dr. Gelles offered opinion testimony that he did "not understand the Schmidt reference to disclose or suggest any assignment of pixels for determining properties of optical signals (or for any other purpose) following determination of the microparticle position." Gelles Decl. ¶ 50. Dr. Gelles's testimony is consistent with the paucity of Schmidt's disclosure on what is meant by "weighted pixel intensity."

Life argues:

> To the extent Patent Owner relies on the Gelles Declaration to argue that Schmidt does not contemplate 'the designation of specific pixels for 'determining characteristics of an optical signal generated at a microparticle'" (PO Response to ACP, pp. 18-19 and Brief, pp. 11-12, citing Declaration at ¶¶ 48-51), that reliance is misplaced for the same reasons detailed above in Section (VII)(A)(1)(b).

Life Resp't Br. 13.

Section (VII)(A)(1)(b) of Life's Respondent Brief addresses the sufficiency of Dr. Gelles Declaration. Life Resp't Br. 7-8. Life contends the

Dr. Gelles's declaration is legally and substantively deficient, and provides no analysis of Brenner to support his conclusion. *Id.* at 7.

We agree with Life that Dr. Gelles's testimony regarding claim 7 is largely opinion-based. However, we have relied on it only to the extent it is consistent with Illumina's arguments that the Examiner's case is deficient.

With respect to the assignment of pixels to microparticles, Life addresses Brenner, but not Schmidt. Life Resp't Br. 8 & 13. Life did not explain how Schmidt's disclosure described a system "configured to designate a first pixel for determining characteristics of an optical signal generated at a microparticle, the first pixel being correlated with the center of the microparticle" as recited in claim 7. Life's arguments are therefore defective for the same reason we found the Examiner's argument deficient.

Accordingly, for the foregoing reasons, we reverse the rejection of claim 7, and dependent claims 8-10 and 13.

3 & 4. OBVIOUSNESS IN VIEW OF BRENNER AND GELLES; BRENNER AND HICKS

Claims 7-10 and 13 stand rejected under 35 U.S.C. § 103 as obvious over Brenner in view of Gelles. Claims 7-12 stand rejected under 35 U.S.C. § 103 as obvious over Brenner in view of Hicks.

The Examiner found that Brenner combined with Gelles or Hicks rendered the subject matter of claim 7, and dependent claims 8-10 and 13 obvious under 35 U.S.C. § 103. RAN 11. The Examiner found:

> Gelles and Hicks also each teach determining the positions of microparticles on a detected image (Gelles, page 450, Figure 1 and corresponding caption; Hicks, page 233, "Microscopy" and

> "Image Analysis," and page 237, Figure 3 and corresponding caption). Gelles and Hicks therefore each teach designating a first pixel for at least determining position characteristics of an optical signal generated at a microparticle, the first pixel corresponding to (or correlated with) the center of the microparticle.

RAN 11-12.

Gelles

Gelles described the movement of beads coated with kinesin on a glass coverslip to which taxol-stabilized microtubules were adhered. Gelles, p. 450, second col., ll. 4-8. Gelles recorded a video of the movement of the beads on the glass. Gelles, p. 450, second col., second paragraph. In the description of Figure 1, Gelles disclosed the methodology to determine the position of the beads on the coverslip. It is true that Gelles describes using *pixels* to determine the position of the beads on the glass slip, but the Examiner did not provide evidence that such description was a disclosure of "designat[ing] a first pixel for determining characteristics of an optical signal generated at a microparticle, the first pixel being correlated with the center of the microparticle" as required by claim 7. As the Examiner did not meet the burden of establishing prima facie obviousness, we are compelled to reverse the rejection of independent claim 7 and dependent claims 8-10 and 13.

Hicks

Hicks tracked movement of latex microspheres (FS) on fibroblasts. Hicks, p. 231. Hicks described using pixel intensities to analyze images. *Id.*

at p. 233 ("Image Analysis"). The Examiner points to this disclosure, but fails to explain how it describes the claimed limitation to "designate a first pixel for determining characteristics of an optical signal generated at a microparticle, the first pixel being correlated with the center of the microparticle" as required by claim 7.

Dr. Gelles testified that he does "not understand Hicks to disclose or suggest a system 'configured to designate a first pixel for determining characteristics of an optical signal generated at a microparticle' as recited in claim 7 (as amended) of the '994 patent." Gelles Decl. ¶ 80. Dr. Gelles supported his opinion with scientific reasoning. *Id.* at ¶¶ 74, 75, 81, and 82. For example, Dr. Gelles explained that Hicks describes "convolution," "a mathematical function that, in essence, compares a group of pixels including and surrounding a microparticle image with a Gaussian (bell) curve. The pixels compared include both pixels with a fluorescent signal and pixels with a low (background) signal." *Id.* at ¶ 74.

Life provides an explanation as to how Hicks is said to meet limitations of dependent claims 11 and 12. Life Resp't Br. 14. However, claims 11 and 12 depend upon claim 7 and insufficient evidence has not been provided that the limitations of claim 7 are met by Hicks.

As the Examiner did not meet the burden of establishing prima facie obviousness, we are compelled to reverse the rejection of independent claim 7 and dependent claims 8-12.

6. OBVIOUSNESS IN VIEW OF BRENNER AND HANSEN

Claims 5 stands rejected under 35 U.S.C. § 103 as obvious in view of Brenner and Hansen. Claim 5 depends on claim 1, and further recites "wherein the coefficient of variation of the diameters of said microparticles is less than two percent." As we reversed the rejection of claim 1, and the Examiner did not provide evidence that Hansen described the deficiencies we found in Brenner, we are compelled to reverse the rejection. See RAN 12; Life Technologies Resp't Br. 15.

CROSS APPEAL

Life appeals the Examiner's determination not to adopt the following rejections:

1. Rejection of claims 25, 31, and 35 under 35 U.S.C. § 102 as anticipated by Brenner.

2. Rejections of claims 29-31 and 33-35 under 35 U.S.C. § 314 as impermissibly enlarging the scope of the claims.

3. Rejection of claims 1-4 under 35 U.S.C. § 102 as anticipated by Lee.[7]

4. Rejection of claims 1, 3, and 4 under 35 U.S.C. § 102 as anticipated by Schmidt.

5. Rejection of claims 1-4 under 35 U.S.C. § 103 as obvious over Gelles.

[7] Ann A. Lee et al. *Biaxial Strain System for Cultured Cells,* U.S. Patent 6,057,150 (granted May 2, 2000) (Lee).

6. Rejection of claim 5 under 35 U.S.C. § 103 as obvious over Lee, Gelles, and Schmidt, in view of Hansen.

7. Various rejections of claims 1-4 under 35 U.S.C. § 103. Life Appeal Br., Ex. A.

8. Rejections of claims 1-4 under 35 U.S.C. § 103 as being obvious over the Brenner, Lee, Schmidt, or Gelles reference in view of the NIH Image Manual.[8]

1. ANTICIPATION BY BRENNER

Claims 25, 31, and 35 each have the limitation that the microparticles are "closely packed." Claim 25 depends on claim 1; claim 31 depends on claim 30, which depends on independent claim 29; claim 35 depends on claim 34, which depends on claim 33, which depends on claim 29.

Each of claims 25, 31, and 35 incorporate the limitation of a "signal tracking means effective to correlate the optical signals from each of the microparticles in each of the sequence of digital images with said center of said microparticle." We found that Brenner does not describe this limitation. Consequently, as dependent claims 25, 31, and 35 each require this limitation, there is sufficient evidence that they are anticipated by Brenner. We thus affirm the Examiner's decision not to adopt the anticipation rejection of claims 25, 31, and 35 over Brenner.

[8] NIH Image Manuel, *NIH Image Version 1.58.*

2. SECTION 314 REJECTION

The Examiner did not adopt the rejection of claims 29-31 and 33-35 under 35 U.S.C. § 314 as impermissibly enlarging the scope of the '994 patent claims. Claim 29 comprises an optical train "configured to collect and focus the sequence of optical signals from the microparticles." The original claims used the language "effective to" rather than "configured to." Life contends that the recitation of "configured to" expands the scope of the '994 patent claims because

> the original claim language, "effective to," could be interpreted as meaning that the claimed structure *actually accomplishes* the recited function, while the new language, "configured to," could be interpreted as meaning merely that the structure is *capable* of performing the recited function.

Life Appeal Br. 15.

We are not persuaded by Life's arguments that the claim language "configured to" recited in claim 29 is broader than the claim language in claim 1 of "effective to." In both cases, the claims are directed to systems "for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps" that comprise an "optical train." The terms "effective to" and "configured to" in each case are followed by the function that the optical train is required to perform in the claimed system. Therefore, a person or ordinary skill in the art would reasonably construe the terms equivalently to indicate that the optical train is capable of performing the recited function. A system is claimed, not a method. So it would be unreasonable to read either "effective to" or "configured to" to further mean that the function must be actually

accomplished. Consequently, we find Life's arguments to have no merit and affirm the Examiner's determination not to adopt the rejection.

3. ANTICIPATION BY LEE

The Examiner did not adopt the rejection of claims 1-4 under 35 U.S.C. § 102 as anticipated by Lee. Illumina distinguished claim 1 from Lee based on the preamble of claim 1. The Examiner adopted Illumina's interpretation. Action Closing Prosecution mailed December 15, 2010 ("ACP") at 28. We find that the Examiner erred in interpreting the claim.

Claim 1 recites in its preamble that the claimed system is "for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps." According to Life, the "sole basis for the Examiner's withdrawal of the rejection of Lee was that 'the microparticles in the system disclosed by Lee are not subject to processing steps as defined by the Bridgham ['994 patent] specification during the observance of the microparticles' as purportedly required by the preamble of claim 1." Life Appeal Br. 21. Life Technologies argues that the preamble phrase "during a sequence of processing steps" is not limiting, and there is no need for Lee to disclose it to anticipate. *Id.*

Illumina contends that the Examiner did not err in not adopting the rejection. Illumina argues:

> Thus, the phrase "sequence of processing steps" [as recited in the claim preamble] is unambiguously descriptive of the "sequence of optical signals" element. (See RAN, at 13). Based on this interpretation, the Examiner correctly determined that the claimed system does not encompass any "system for detecting a sequence of optical signals from each of a plurality

> of microparticles," that may be operated "during a sequence of (generic) processing steps." (RAN, at 13). Although the entire phrase "detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps" does not appear in the body of claim 1, the elements of "the sequence of optical signals from the microparticles," "said signals," and "the optical signals from each of the microparticles" each refer to the optical signals that are generated during, and as a result of, the "sequence of processing steps."

Illumina Resp't Br. 7.

Legal Principles

"Preamble language that merely states the purpose or intended use of an invention is generally not treated as limiting the scope of the claim." *Bicon, Inc. v. Straumann Co.*, 441 F.3d 945, 951 (Fed. Cir. 2006).

> If the body of the claim 'sets out the complete invention,' the preamble is not ordinarily treated as limiting the scope of the claim. *Schumer v. Lab. Computer Sys., Inc.*, 308 F.3d 1304, 1310 (Fed. Cir. 2002)). However, the preamble is regarded as limiting if it recites essential structure that is important to the invention or necessary to give meaning to the claim. [citations omitted]. . . When limitations in the body of the claim rely upon and derive antecedent basis from the preamble, then the preamble may act as a necessary component of the claimed invention." *Eaton Corp. v. Rockwell Int'l Corp.*, 323 F.3d 1332, 1339 (Fed. Cir. 2003).

Id. at 952.

Issue

The issues in this rejection are thus: is the invention set forth in the body of the claim is complete or does the claimed invention require the

preamble limitation "during a sequence of processing steps" for completeness; and is the preamble limitation is merely a "purpose or intended use" of the claimed invention?

Discussion

The claimed "optical train," "imaging device," and "signal tracking means" each have recited functions. These components are not simply general purpose structures, but must be "effective" or have the ability to carry out the recited function. See Claim interpretation, supra at p. 9. With respect to the "optical train," the claim requires it to be able "to collect and focus the sequence of optical signals from the microparticles, and to record at least one optical characteristic of each microparticle." The "imaging device" must be able to "generate and record a sequence of digital images of the microparticles" comprising the optical signals. The "signal tracking means must "correlate the optical signals from each of the microparticles in each of the sequence of digital images with said center of said microparticle." These functions are express limitations that constrain the "optical train," "imaging device," and "signal tracking means" to structures which are able to perform the recited functions. The components must therefore be capable of capturing sequences of signals and sequences of digital images.

The claim preamble that the signals and images are captured "during a sequence of processing steps" does not further limit or define the subsequently recited structures "optical train," "imaging device," and "signal tracking means," but rather simply reflects the intention that the system is

used during such processing steps. The processing steps constitute an environment in which the system is capable of being used, and is intended to be used, but it does not change the structure or capability of the subsequently recited components. In fact, there is no component recited in the body of the claim which would perform the sequence of processing steps. Thus, we do not see, and Illumina has not adequately explained, how the claimed preamble is a necessary component of the claimed invention and necessary to complete the invention recited in the claim body.

In contrast, claim 29 which has similar language adds the additional limitation of "a fluidics controller effective to deliver reagents to the flow cell for a sequence of processing steps." Absent express language in the claim, we will not read this imitation into claim 1. Life Appeal Br. 19-20.

For the foregoing reasons, we conclude that the Examiner erred in not adopting the rejection of claims 1-4 as anticipated by Lee.

4. ANTICIPATION BY SCHMIDT

Life proposed a rejection of claims 1, 3, and 4 under 35 U.S.C. § 102 as anticipated by the Schmidt reference which the Examiner initially adopted, but subsequently withdrew. ACP 31-32.

Schmidt described the movement of colloidal gold particles on cells. Schmidt, p. 977. The gold particles were coated with anti-B1 integrin antibody. *Id.* The antibody bound to integrin on the cells, attaching the colloidal gold particles to the cell surface. *Id.* "Small gold aggregates [attached to the antibody] were rapidly transported preferentially to the leading edge of the lamellipod where they resumed diffusion restricted along

the edge." *Id.* The transport of the colloidal gold particles on the cells was tracked using video microscopy. *Id.* The Examiner found that "Schmidt does not disclose a planar array of uniformly sized spherical microparticles" as recited in independent claim 1. ACP 31-32.

Life contends that "Schmidt does disclose such a planar array in that Schmidt detects and monitors the displacements of microparticles in an array on a substantially planar surface of cultured adherent cells on a glass slide, where microbeads are placed into contact with the cells. Indeed, Schmidt *discloses two planar* arrays, one comprising cells on the glass slide and one comprising microbeads, smaller in scale than the cells." Life Appeal Br. 22.

These arguments are not persuasive. First, Life has not provided persuasive evidence that cells are microparticles as that term would be interpreted in light of the '994 patent specification. Secondly, Life has not explained how colloidal particles distributed on the three-dimensional surface of cells constitutes a planar array as that term would be construed in light of the patent. Consequently, we agree with Illumina that the Examiner did not err in not adopting the rejection of claims 1, 3, and 4 as anticipated by Schmidt.

5. OBVIOUSNESS IN VIEW OF GELLES

Life proposed a rejection of claims 1-4 under 35 U.S.C. § 103 as obvious over Gelles which the Examiner initially adopted, but subsequently withdrew. ACP 36-37.

Gelles describes a system for recording, analyzing, and determining precise positional information of microscopic plastic beads (i.e.,

microparticles) on a glass coverslip (i.e., a planar array) as the microparticles, driven by kinesin, attach to and move along microtubules *in vitro* (i.e., a sequence of processing steps). Gelles, p. 450, abstract and column 2, first paragraph, through page 451, first partial paragraph; and Fig. 2).

Life contends that the Examiner did not adopt the rejection because Gelles did not subject the microparticles to processing steps and because Gelles did not disclose uniform particles, both of which the Examiner stated are required by independent claim 1. Life Appeal Br. 23; *see* ACP 36-37. Illumina contends that Gelles does not disclose a sequence of processing steps. Illumina Resp't Br. 10.

As discussed above, the preamble phrase "during a sequence of processing steps" is not limiting, and there is no need for Gelles to disclose it to anticipate or render the claim obvious. Because the Examiner erred in interpreting the claim and Illumina has not otherwise distinguished the claim over Gelles, we find that the Examiner erred in withdrawing the rejection of claims 1-4 as obvious in view of Gelles.

6. OBVIOUSNESS REJECTIONS OVER LEE, SCHMIDT, AND GELLES IN VIEW OF HANSEN

Life proposed a rejection of claim 5 under 35 U.S.C. § 103 as obvious over Lee, Gelles, and Schmidt, in view of Hansen. Life contends the Examiner

> declined to adopt obviousness rejections based on Lee, Schmidt or Gelles, in view of Hansen solely because the Examiner found that Lee, Schmidt, and Gelles each purportedly fail to anticipate

> and/or render independent claim 1 obvious. *See* ACP, pp. 20-21, 31, 35-36, and 41. As demonstrated above, the Examiner should not have withdrawn the rejections of claim 1 in view of Lee, Schmidt, and Gelles. Because claim 1 is anticipated by and/or obvious over each of Lee, Schmidt, and Gelles, the Examiner also erred in failing to adopt the above-identified rejections of claim 5.

Life Appeal Br. 24.

We concluded that the Examiner erred in not adopting the rejections of the claims as anticipated by the Lee and Gelles publications. However, we concluded that the Examiner properly determined that the claims were not anticipated by Schmidt. Illumina did not dispute that Hansen in view of Lee and Gelles described or suggested the subject matter of claim 5. Illumina Resp't Br. 10. Therefore, we shall reverse the Examiner's determination not to adopt the obviousness rejection of claim 5 over Lee and Gelles in view of Hansen, but affirm as it pertains to Schmidt.

7. VARIOUS NON-ADOPTED OBVIOUSNESS REJECTIONS

Life proposed numerous rejections of claims 1-4 under 35 U.S.C. § 103 that Life contends the Examiner did not consider because it "it would be 'improper' to enter a multiple reference obviousness rejection where a single reference obviousness rejection over the same reference had already been applied." Life Appeal Br. 26. Life contends that the "Examiner's refusal to adopt those obviousness SNQs solely because an anticipation or obviousness rejection over the same reference had already been applied is not legally tenable, is contrary to the MPEP, and is in tension with the prohibition against piecemeal prosecution." *Id.*

In the Request for Reexamination, Life proposed numerous rejections under 35 U.S.C. § 103. For example Gelles was combined with each of the following: Brenner, Lee, Schmidt, Douglass, Stern, King, Luck, Sizto, DiMilla, Dow, Wernet, Wilson, Brandriss, NIH Image Manual, Brenner & Brandriss, Lee & Brandriss, Schmidt & Brandriss, or Douglas & Brandriss, in eighteen separate rejections. The Examiner indicated that the rejections raised a substantial new question of patentability. Decision Granting Inter Partes Reexamination, May 10, 2010, p. 4-6. However, in a subsequent Office Action, the Examiner did not adopt all of them because the Examiner found that Gelles already described the limitations for which the additionally secondary references were cited. Non-Final Office Action, May 10, 2010, p. 13.

We agree with the Examiner. Relying on 18 separate rejections for the same limitations, and for the same limitations already identified in the primary reference, is cumulative and unnecessarily duplicative. The limitations were addressed by the Examiner in adopted rejections, making the non-adopted rejections moot.

Moreover, had Life believed that the adopted rejections were inadequate, Life could have pointed out the differences in the proposed rejections to the Examiner, and explained why they were necessary to establish unpatentability. Under 35 U.S.C. § 314(c), inter partes reexaminations are to be conducted with "special dispatch." Cumulative rejections add to the time it takes to conduct an appeal, and thus limiting the issues is important to comply with 35 U.S.C. § 314(c).

8. OBVIOUSNESS IN VIEW OF NIH IMAGE MANUAL

The Examiner declined to consider Requester's proposed rejections based on the NIH Image Manual because Life did not establish that it qualified as prior art against the '994 patent. ACP 42-43; RAN 15. Life Challenges this determination. Life contends:

> Requester has established, at least *prima facie*, that the manual was available at the same time as the software. As explained in the previously-submitted 1995 Rasband Reference (*see* Requester's August 9, 2010 submission, p. 24), NIH Image software is accompanied by a user manual. Thus, given that the 1995 Rasband Reference explains that NIH Image software is accompanied by a user manual, the NIH Image software referenced in the 1995 Opalenik Reference would have included the associated user manual. In the absence of any evidence to the contrary, and in light of the evidentiary standard for demonstrating that a reference is prior art, Requester has adequately established that the NIH Image Manual v. 1.58 is prior art.

Life Appeal Br. 28.

Legal Principles

> "Because there are many ways in which a reference may be disseminated to the interested public, 'public accessibility' has been called the *touchstone* in determining whether a reference constitutes a 'printed publication' bar under 35 U.S.C. §102(b)." *In re Hall*, 781 F.2d 897, 898-99 (Fed. Cir. 1986) (emphasis added). "A given reference is 'publicly accessible' upon a satisfactory showing that such document has been disseminated or otherwise made available to the extent that persons interested and ordinarily skilled in the subject matter or art exercising reasonable diligence, can locate it." *Bruckelmyer v. Ground Heaters, Inc.*, 445 F.3d 1374, 1378 (Fed. Cir. 2006).

SRI International Inc. v. Internet Security Systems Inc., 511 F.3d 1186, 1194 (Fed. Cir. 2008).

Discussion

The Examiner did not accept Life's arguments that the NIH Image Manual was prior art because the Manual did not have publication date (Non-Final Office Action, dated May 10, 2010, p. 12). Life responded by providing a publication by Rasband and Bright (*Microbeam Analysis*, 4:137-149 (1995)) which disclosed: "NIH Image comes with many other files: a user manual 'About NIH Image [.]'" Rasband, p.137, second col, first full paragraph. However, Life did not establish the document submitted by them titled "NIH Image (Version 1.58)" is the same manual referred to by Rasband and Bright. The evidence provided by Life is insufficient to establish that NIH Image (Version 1.58) was publically accessible prior to the effective filing date of the '994 Patent.

SUMMARY

Appeal

1. The rejection of claims 1-4, 6, 14-24, 26-30, 33, and 34 as anticipated by Brenner is reversed.

2. The rejection of claims 7-10 and 13 as obvious over Brenner in view of Schmidt is reversed.

3. The rejection of claims 7-10 and 13 as obvious over Brenner in view of Gelles is reversed.

4. The rejection of claims 7-12 as obvious over Brenner in view of Hicks is reversed.

5. The rejection of claim 5 as obvious over Brenner in view of Hansen is reversed.

Cross-Appeal

1. The Examiner's determination not to adopt the rejection of claims 25, 31, and 35 under 35 U.S.C. § 102 as anticipated by Brenner is affirmed.

2. The Examiner's determination not to adopt the rejection of claims 29-31 and 33-35 under 35 U.S.C. § 314 as impermissibly enlarging the scope of the claims is affirmed.

3. The Examiner's determination not to adopt the rejection of claims 1-4 under 35 U.S.C. § 102 as anticipated by Lee is reversed.

4. The Examiner's determination not to adopt the rejection of claims 1, 3, and 4 under 35 U.S.C. § 102 as anticipated by Schmidt is affirmed.

5. The Examiner's determination not to adopt the rejection of claims 1-4 under 35 U.S.C. § 103 as obvious over Gelles is reversed.

6. The Examiner's determination not to adopt the rejection of claim 5 under 35 U.S.C. § 103 as obvious over Lee and Gelles in view of Hansen is reversed.

7. The Examiner's determination not to adopt the rejection of claim 5 under 35 U.S.C. § 103 as obvious over Lee, Gelles, and Schmidt in view of Hansen is reversed.

8. The Examiner's determination not to adopt various obviousness rejections under 35 U.S.C. § 103 is affirmed.

9. The Examiner's determination not to adopt the rejection of claims 1-4 under 35 U.S.C. § 103 as obvious over the Brenner, Lee, Schmidt, or Gelles reference in view of the NIH Image Manual is affirmed.

NEW GROUNDS OF REJECTION

37 C.F.R. § 41.77(a) states that "[t]he reversal of the examiner's determination not to make a rejection proposed by the third party requester constitutes a decision adverse to the patentability of the claims which are subject to that proposed rejection which will be set forth in the decision of the Board of Patent Appeals and Interferences as a new ground of rejection." Accordingly, for the reasons given above, we enter the following new grounds of rejection:

Claims 1-4 are rejected under 35 U.S.C. § 102 as anticipated by Lee.

Claims 1-4 are rejected under 35 U.S.C. § 103 as obvious over Gelles.

Claim 5 is rejected under 35 U.S.C. § 103 as obvious over Lee and Gelles in view of Hansen.

Claim 5 is rejected under 35 U.S.C. § 103 as obvious over Lee, Gelles, and Schmidt in view of Hansen

This decision contains new grounds of rejection pursuant to 37 C.F.R. § 41.77(b) which provides that "[a]ny decision which includes a new ground of rejection pursuant to this paragraph shall not be considered final for judicial review." Accordingly, no portion of the decision is final for purposes of judicial review. A requester may also request rehearing under 37 C.F.R. § 41.79, if appropriate, however, the Board may elect to defer issuing any decision on such request for rehearing until such time that a final decision on appeal has been issued by the Board.

For further guidance on new grounds of rejection, see 37 C.F.R. § 41.77(b)-(g). The decision may become final after it has returned to the Board. 37 C.F.R. § 41.77(f).

37 C.F.R. § 41.77(b) also provides that the Patent Owner, WITHIN ONE MONTH FROM THE DATE OF THE DECISION, must exercise one of the following two options with respect to the new grounds of rejection to avoid termination of the appeal as to the rejected claims:

(1) *Reopen prosecution.* The owner may file a response requesting reopening of prosecution before the examiner. Such a response must be either an amendment of the claims so rejected or new evidence relating to the claims so rejected, or both.

(2) *Request rehearing.* The owner may request that the proceeding be reheard under § 41.79 by the Board upon the same record. …

Any request to reopen prosecution before the examiner under 37 C.F.R. § 41.77(b)(1) shall be limited in scope to the "claims so rejected." Accordingly, a request to reopen prosecution is **limited** to issues raised by the new ground(s) of rejection entered by the Board. A request to reopen prosecution that includes issues other than those raised by the new ground(s) is unlikely to be granted. Furthermore, should the patent owner seek to substitute claims, there is a presumption that only one substitute claim would be needed to replace a cancelled claim.

A requester may file comments in reply to a patent owner response. 37 C.F.R. § 41.77(c). Requester comments under 37 C.F.R. § 41.77(c) shall be **limited** in scope to the issues raised by the Board's opinion reflecting its decision to reject the claims and the patent owner's response under paragraph 37 C.F.R. § 41.77(b)(1). A newly proposed rejection is not permitted as a matter of right. A newly proposed rejection may be appropriate if it is presented to address an amendment and/or new evidence properly submitted by the patent owner, and is presented with a brief explanation as to why the newly proposed rejection is now necessary and why it could not have been presented earlier.

Compliance with the page limits pursuant to 37 C.F.R. § 1.943(b), for all patent owner responses and requester comments, is required.

The examiner, after the Board's entry of a patent owner response and requester comments, will issue a determination under 37 C.F.R. § 41.77(d) as to whether the Board's rejection is maintained or has been overcome. The proceeding will then be returned to the Board together with any comments and reply submitted by the owner and/or requester under 37 C.F.R. § 41.77(e) for reconsideration and issuance of a new decision by the Board as provided by 37 C.F.R. § 41.77(f).

Requests for extensions of time in this *inter partes* reexamination proceeding are governed by 37 C.F.R. § 1.956. *See* also 37 C.F.R. § 41.79.

Appeal 2012-010385
Reexamination Control No. 95/000,529
US Patent 6,831,994 B2

REVERSED; NEW GROUNDS UNDER § 41.77(b)

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
95/000,529	02/19/2010	6831994	048522-0003	8420

22922 7590 10/29/2013

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EXAMINER	
LEUNG, CHRISTINA Y	
ART UNIT	**PAPER NUMBER**
3992	

MAIL DATE	DELIVERY MODE
10/29/2013	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Illumina, Inc.
(Patent Owner and Appellant)

v.

Life Technologies Corporation
(Requester and Cross-Appellant)

Appeal 2012-010385
Reexamination Control No. 95/000,529
US Patent 6,831,994 B2
Technology Center 3900

Before RICHARD M. LEBOVITZ, JEFFREY B. ROBERTSON, and RAE LYNN P. GUEST, *Administrative Patent Judges*.

LEBOVITZ, *Administrative Patent Judge*.

DECISION ON REHEARING

Patent Owner requests rehearing under 37 C.F.R. § 41.77(b)(2) of the PTAB Decision dated February 26, 2013 ("Decision") in the appeal from the Examiner's decision in the above-identified *inter partes* reexamination of US 6,831,994 ("the '994 Patent") (Request for Rehearing, dated March 26, 2013 (Req. Reh'g)).

In the Decision, we reversed the Examiner's determination not to adopt the following rejections:

1. Claims 1-4 under 35 U.S.C. § 102 as anticipated by Lee.

2. Claims 1-4 under 35 U.S.C. § 103 as obvious over Gelles.

3. Claim 5 under 35 U.S.C. § 103 as obvious over Lee and Gelles in view of Hansen.

4. Claim 5 under 35 U.S.C. § 103 as obvious over Lee, Gelles, and Schmidt in view of Hansen.

The four reversals were based upon our finding that the Examiner erred in interpreting claim 1 (Decision 21 and 26). Specifically, we found that the preamble of claim 1 reciting "during a sequence of processing steps" in the claimed system "for detecting a sequence of optical signals from each of a plurality of microparticles" does not further limit the claim nor distinguish the claim from the systems described in the Lee and Gelles publications. Rather, we found the preamble language "during a sequencing of processing steps" constitutes "an environment in which the system is capable of being used, and is intended to be used, but [the preamble language] does not change the structure or capability of the subsequently recited components." (*Id.* at 24.)

Patent Owner contends that our analysis "focuses solely on the language of the claim and does not evaluate any description in the '994 patent outside of the claims, or consider the prosecution history of the '994 patent." (Req. Reh'g 7.) Patent Owner argues that the structure of the claim shows that the preamble was intended to be limiting.

> The "optical train" in the body of claim 1 refers to the element "the sequence of optical signals." The only antecedent basis for "the sequence of optical signals" is from the preamble limitation "a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps." In other words, the claim itself makes clear in the body that it is not referring to just any optical signals, but rather the optical signals obtained during the sequence of processing steps referred to in the preamble . . . .

(*Id.* at 9.) This intent, according to Patent Owner, is reflected in the '994 Patent specification which is said to repeatedly describe obtaining signals during a sequence of processing steps (*id.*)

We agree with Patent Owner that the '994 Patent teaches that the claimed system was designed to handle microparticles through a series of processing steps and collect optical signals from the microparticles during these steps. For example, the inventors stated in the '994 Patent:

> It would be especially desirable if such system and apparatus permitted the tracking and analysis of multiple analytes anchored to separate microparticles through a sequence of several processing and/or analysis steps.

(Col. 1, ll. 60-63.) The '994 Patent describes a method of loading microparticles into a flow chamber, subjecting the microparticles to a sequence of steps in which liquid reagents are sequentially delivered to the flow chamber (col. 5, ll. 37-44; col. 8, ll. 4-13), and collecting optical signals

and images during the sequence of steps (col. 5, ll. 4-11 and 44-47; col. 10, ll. 13-38). However, claim 1 is directed to a system. A system is described in the '994 Patent to include an apparatus (which comprises a flow chamber, fluidic means, and detection means), microparticles, and software means for analyzing optical signals (col. 2, ll. 20-38). The system is utilized to collect a sequence of optical signals during a "sequence of processing steps," but the system itself is not drawn to a method comprising the steps. For this reason, we do not understand how it could be proper to insert a method step into a system claim.

The system is recited in claim 1 to be "for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps." The system is recited in the claim to comprise an "optical train," "imaging device," and "signal tracking means," each of which relate to the detection of "optical signals" from the plurality of microparticles" recited in the claim preamble. However, the limitation that the optical signals are detected in a "sequence of processing steps" has no corresponding structure in the claim.

For example, in the patent, the processing steps are performed in a flow chamber and reagents are delivered by a fluidic means:

> Loaded microparticles are disposed in a planar array in a flow chamber through which processing reagents are sequentially delivered to the loaded microparticles from one or more reagent reservoirs by a fluidic means. Optical signals generated by, or produced as a result of, the interaction of processing reagents and polynucleotides on the microparticles are imaged by a detection means.

(Col. 2, ll. 45-51.) These elements, however, are not recited in the claim.

It is simply not clear how a "sequence of processing steps" would further limit a "system." A "step" is an act, while a system is a structure comprising the means for performing the act. Asking us to conclude that the phrase "sequence of processing steps" limits the scope of claim 1 is either asking us to read a process step in a system claim or to import a structure in the system for performing the processing steps. The Federal Circuit has held that language in an apparatus or product claim directed to the function, operation, intent-of-use, and materials upon which the components of the structure work that does not structurally limit the components or patentably differentiate the claimed apparatus or product from an otherwise identical prior art structure will not support patentability. *See, e.g.*, *In re Rishoi*, 197 F.2d 342, 344-45 (CCPA 1952); *In re Otto*, 312 F.2d 937, 939-40 (CCPA 1963); *In re Ludtke*, 441 F.2d 660, 663-64 (CCPA 1971); *In re Yanush*, 477 F.2d 958, 959 (CCPA 1973). Patent Owner has not articulated a reason as to why a system claim should be read to require a step to be performed in it. Patent Owner also has not identified any specific structure required by the system for performing the processing step function.

As stated in the Decision, claim 29, which is directed to a system for "for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps," comprises a flow cell in which the processing steps are carried out and "a fluidics controller effective to deliver reagents to the flow cell for a sequence of processing steps." However, neither structure is present in claim 1.

Patent Owner also argues that the importance of the preamble as limiting claim 1 was confirmed during the original prosecution of the '994 Patent. Patent Owner states:

> The patentee emphasized the importance of the sequence of processing steps to the claimed system, stating "an important advantage of the invention is its 'ability to keep track of individual microparticles through multiple process steps and/or cycles.'" (December 19, 2003, Response, p. 6.)

(Req. Reh'g 10.)

This statement during prosecution of the '994 Patent does not explain how "sequence of processing steps" limits the claim. To the contrary, the statement that the claimed system is capable of keeping track of microparticles is reflected in the recited system components of an optical train, imaging device, and signal tracking means.

For the foregoing reasons, we decline to modify our decision reversing the Examiner's determination to adopt rejections 1-4 listed above.

REHEARING DENIED

Appeal 2012-010385
Reexamination Control No. 95/000,529
US Patent 6,831,994 B2

ack

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US006831994B2

(12) **United States Patent**
Bridgham et al.

(10) **Patent No.: US 6,831,994 B2**
(45) **Date of Patent: Dec. 14, 2004**

(54) **SYSTEM AND APPARATUS FOR SEQUENTIAL PROCESSING OF ANALYTES**

(75) Inventors: **John Bridgham**, Hillsborough, CA (US); **Kevin Corcoran**, Fremont, CA (US); **George Golda**, El Granada, CA (US); **Michael C. Pallas**, San Bruno, CA (US); **Sydney Brenner**, La Jolla, CA (US)

(73) Assignee: **Lynx Therapeutics, inc.**, Hayward, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 196 days.

(21) Appl. No.: **09/908,130**

(22) Filed: **Jul. 17, 2001**

(65) **Prior Publication Data**

US 2002/0051992 A1 May 2, 2002

Related U.S. Application Data

(62) Division of application No. 09/424,028, filed as application No. PCT/US98/11224 on May 22, 1998, now Pat. No. 6,406,848, which is a continuation of application No. 08/862,610, filed on May 23, 1997, now abandoned.

(51) **Int. Cl.**[7] **G06K 9/00**

(52) **U.S. Cl.** **382/133**; 250/461.2

(58) **Field of Search** 382/100, 128, 382/129–134, 181, 278; 250/458.1, 461.2; 356/317, 318; 359/225, 368; 422/131, 165; 435/6, 69.1; 436/518; 514/12

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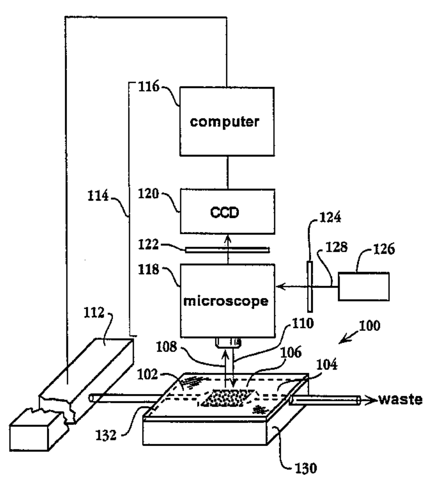
Primary Examiner—Jayanti K. Patel
(74) *Attorney, Agent, or Firm*—Vincent M. Powers; Lee Ann Gorthey; Perkins Coie LLP

(57) **ABSTRACT**

An apparatus and system are provided for simultaneously analyzing a plurality of analytes anchored to microparticles. Microparticles each having a uniform population of a single kind of analyte attached are disposed as a substantially immobilized planar array inside of a flow chamber where steps of an analytical process are carried out by delivering a sequence of processing reagents to the microparticles by a fluidic system under microprocessor control. In response to such process steps, an optical signal is generated at the surface of each microparticle which is characteristic of the interaction between the analyte carried by the microparticle and the delivered processing reagent. The plurality of analytes are simultaneously analyzed by collecting and recording images of the optical signals generated by all the microparticles in the planar array. A key feature of the invention is the correlation of the sequence of optical signals generated by each microparticle in the planar array during the analytical process.

4 Claims, 10 Drawing Sheets



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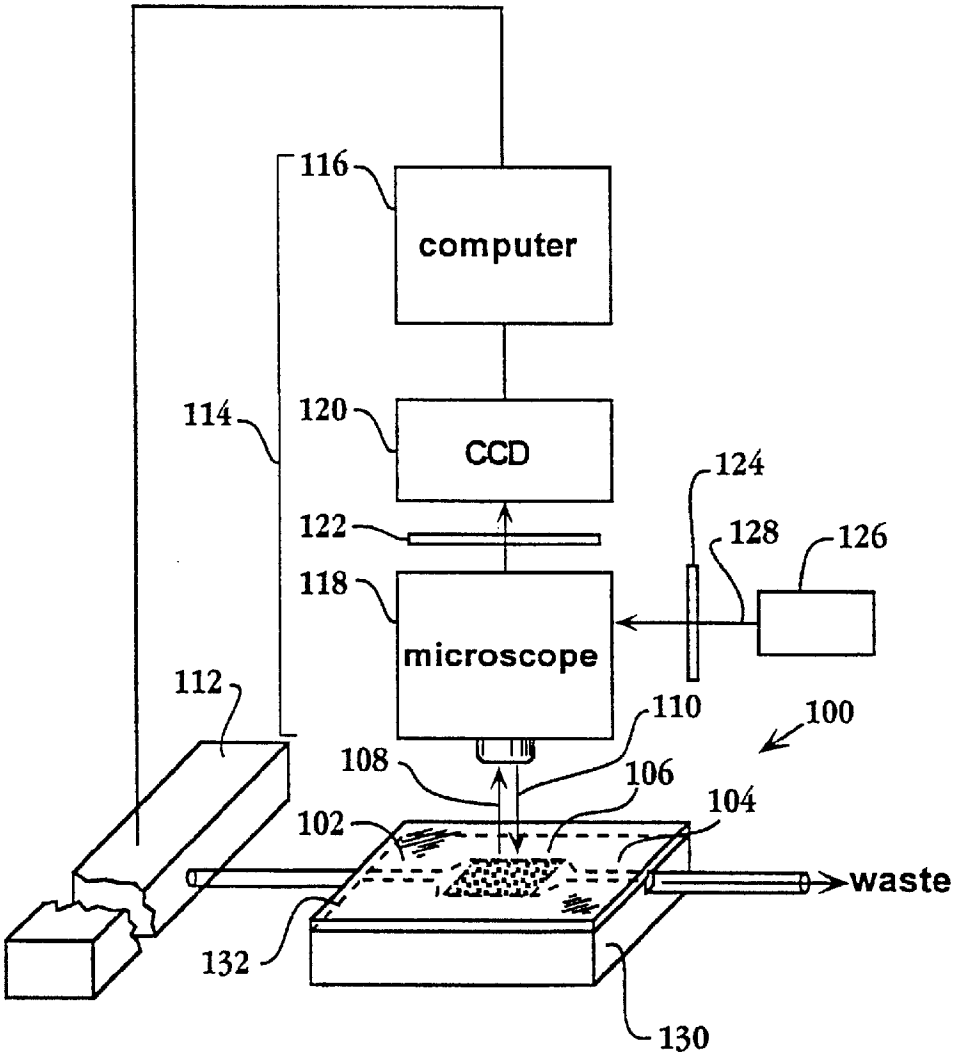
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Fig. 1A

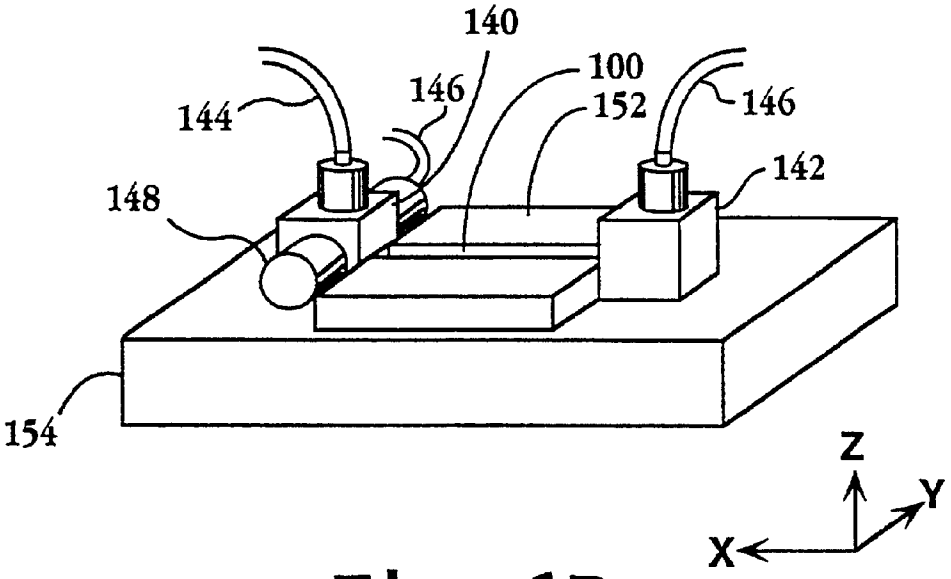

Fig. 1B

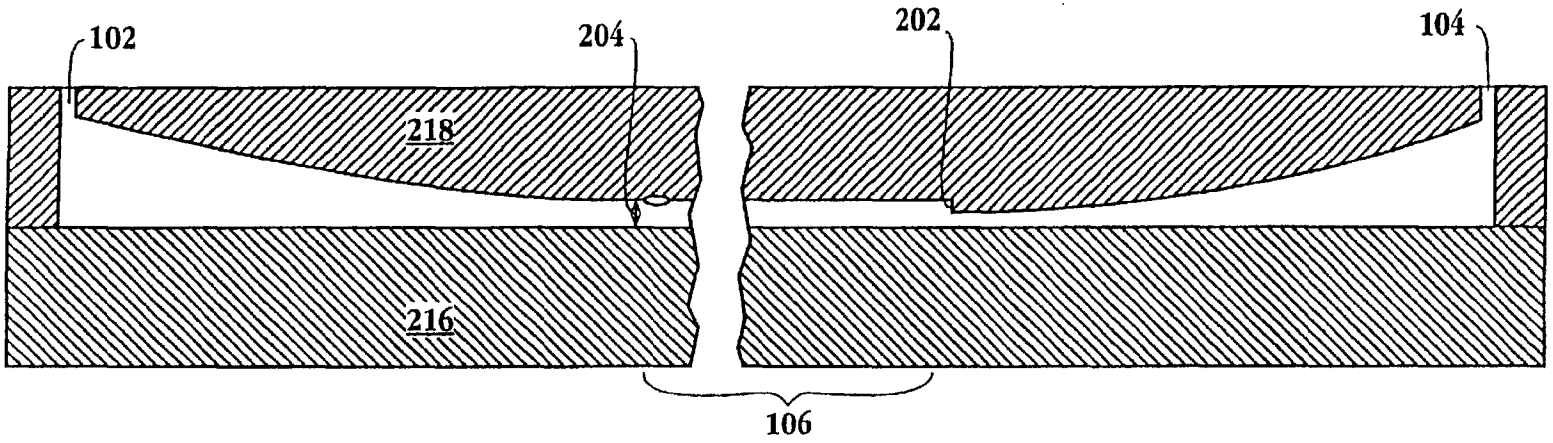
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Fig. 2A

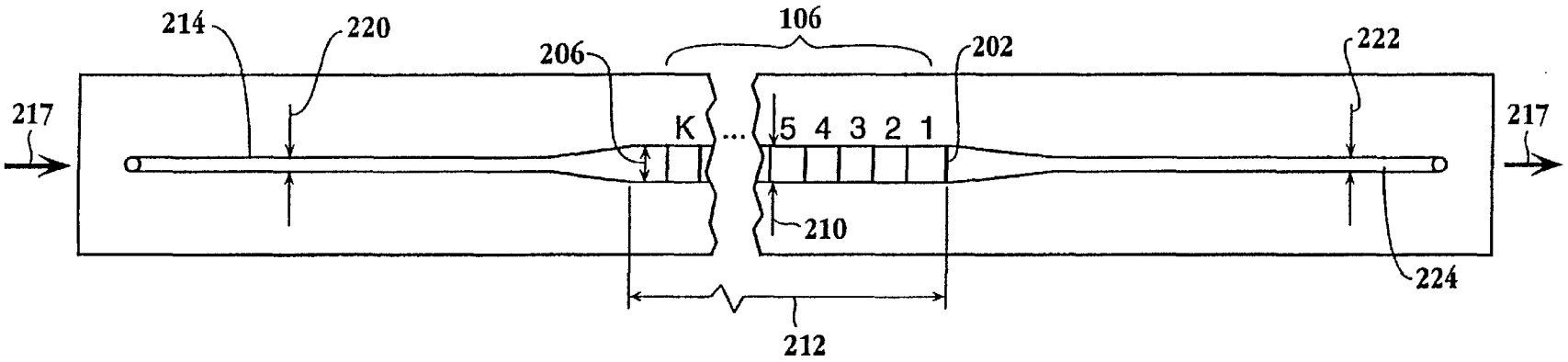
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Fig. 2B

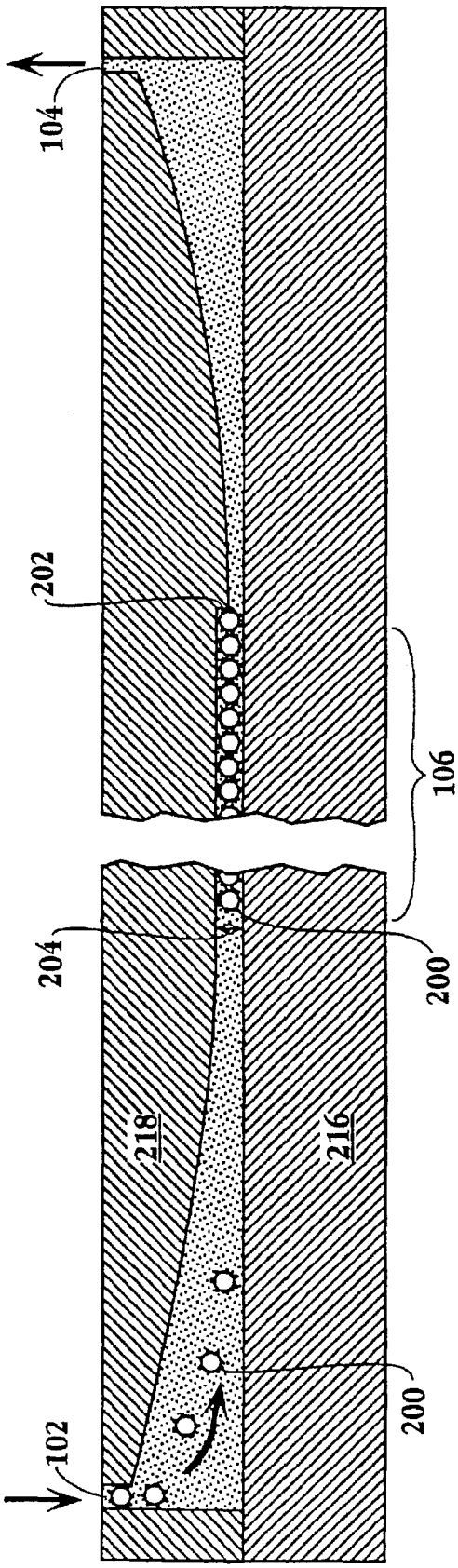

Fig. 2C

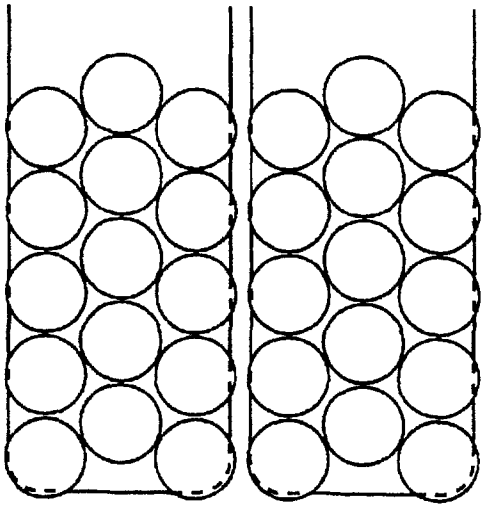

Fig. 3A

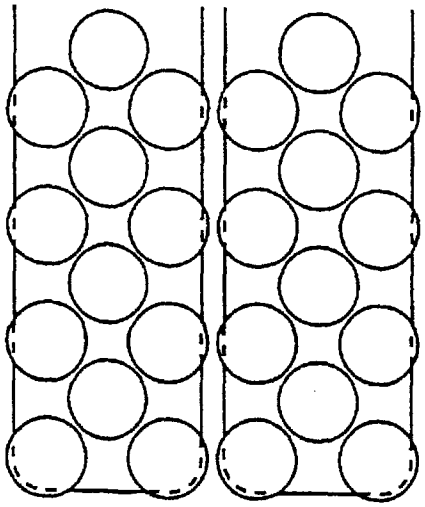

Fig. 3B

Fig. 3C

Fig. 3D

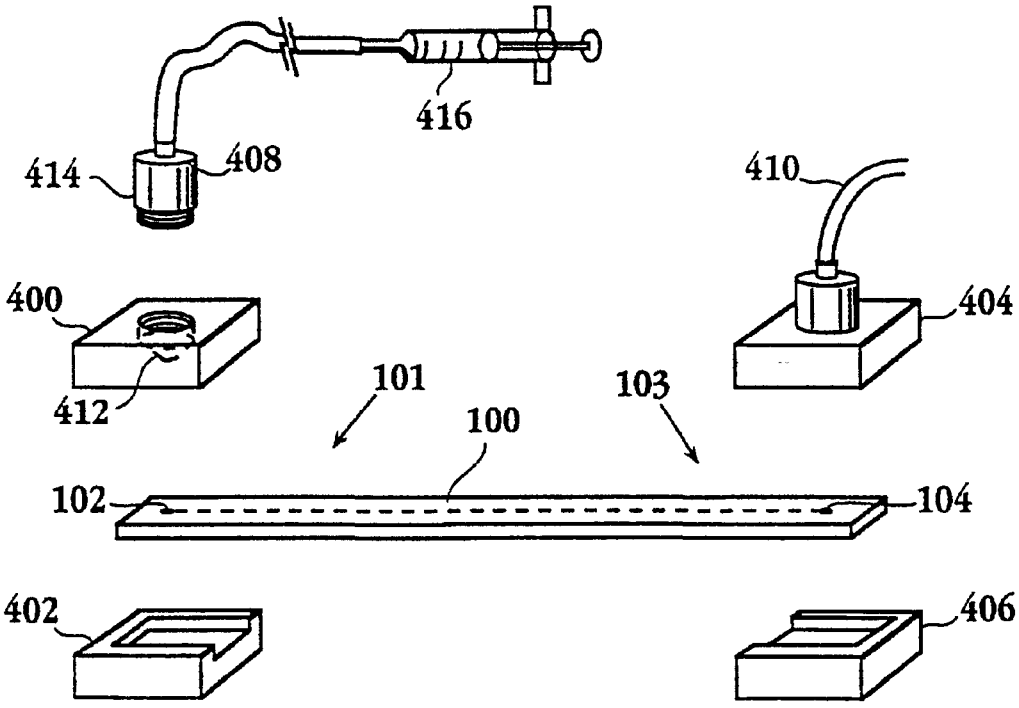

Fig. 4

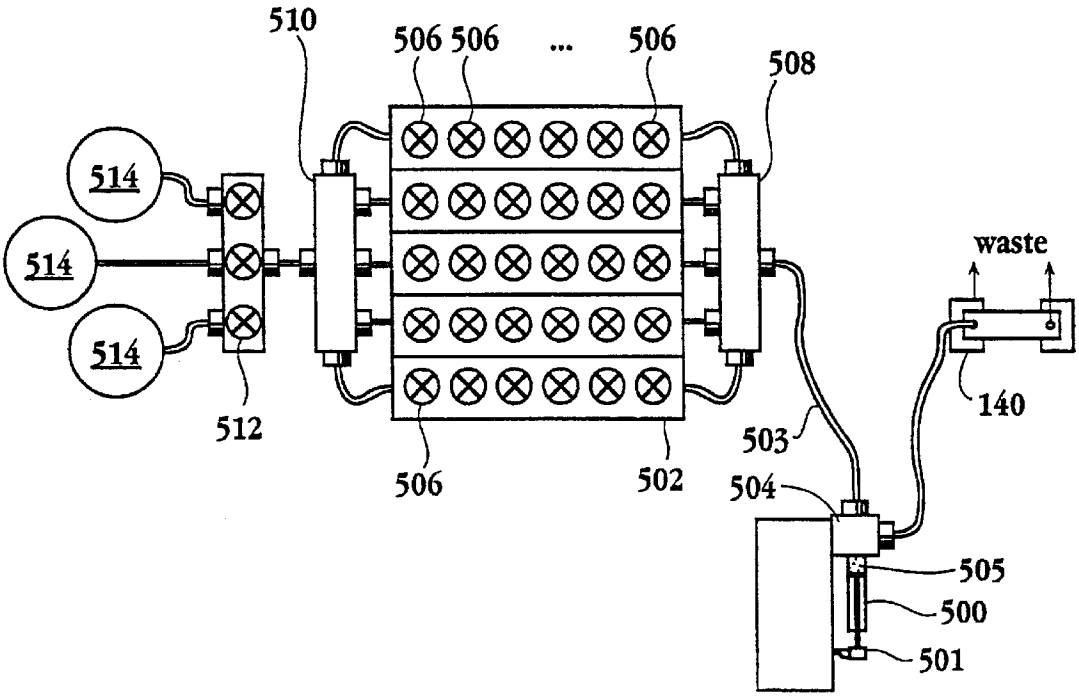
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Fig. 5

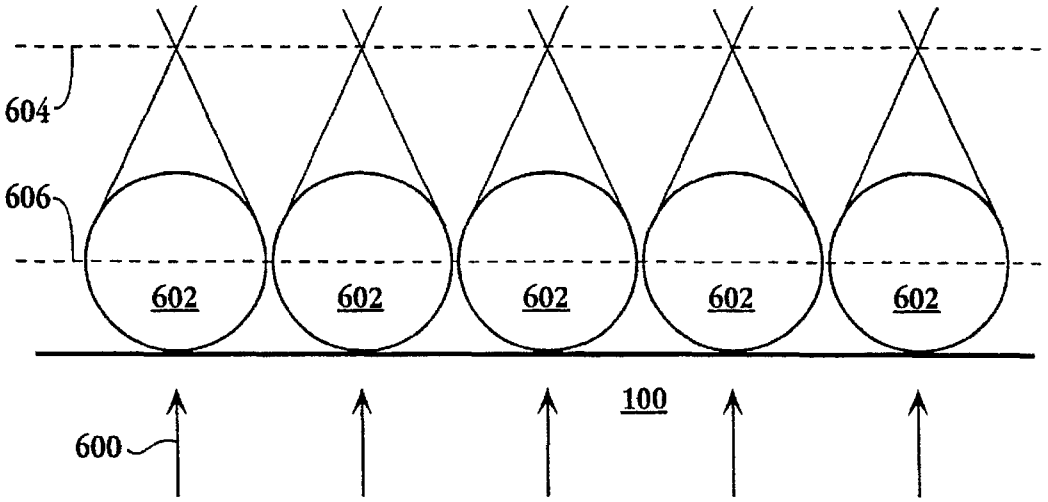
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Fig. 6A

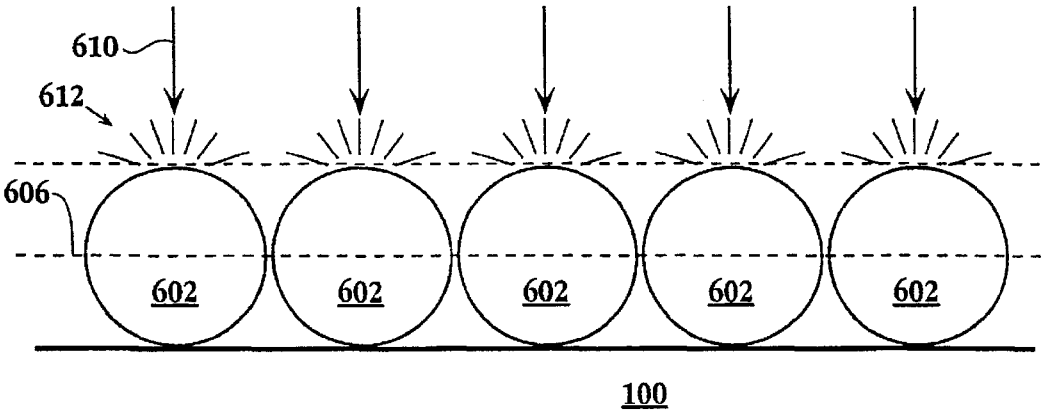
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Fig. 6B

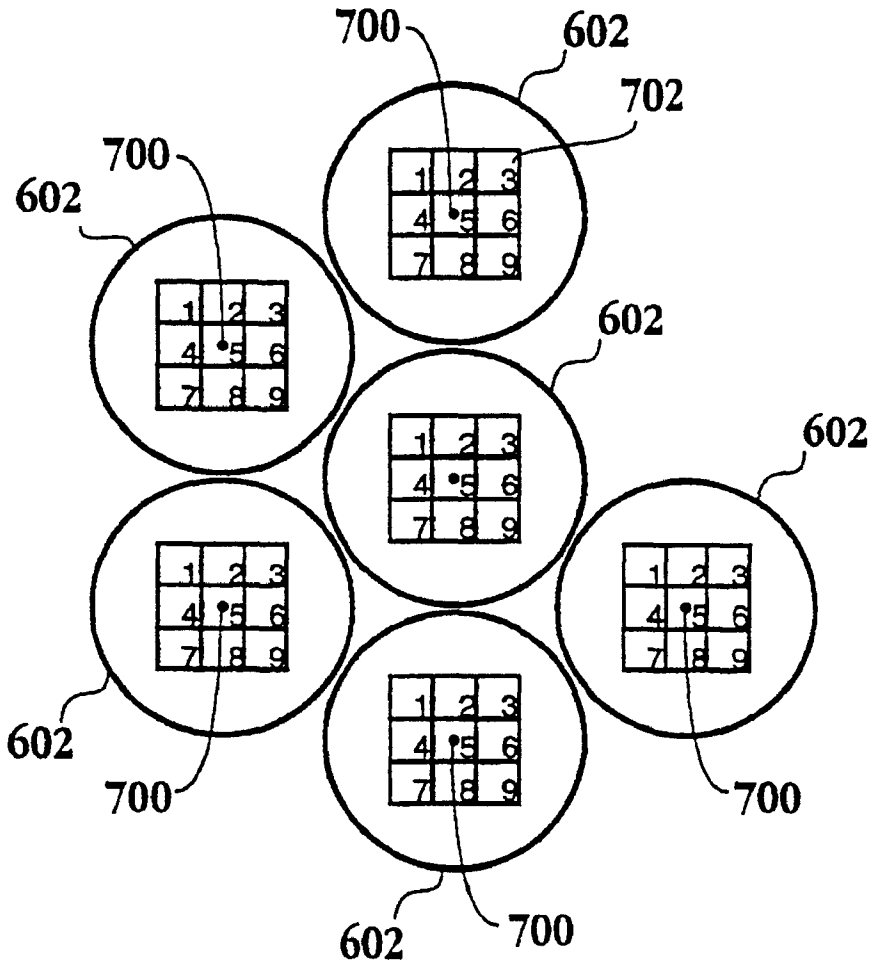
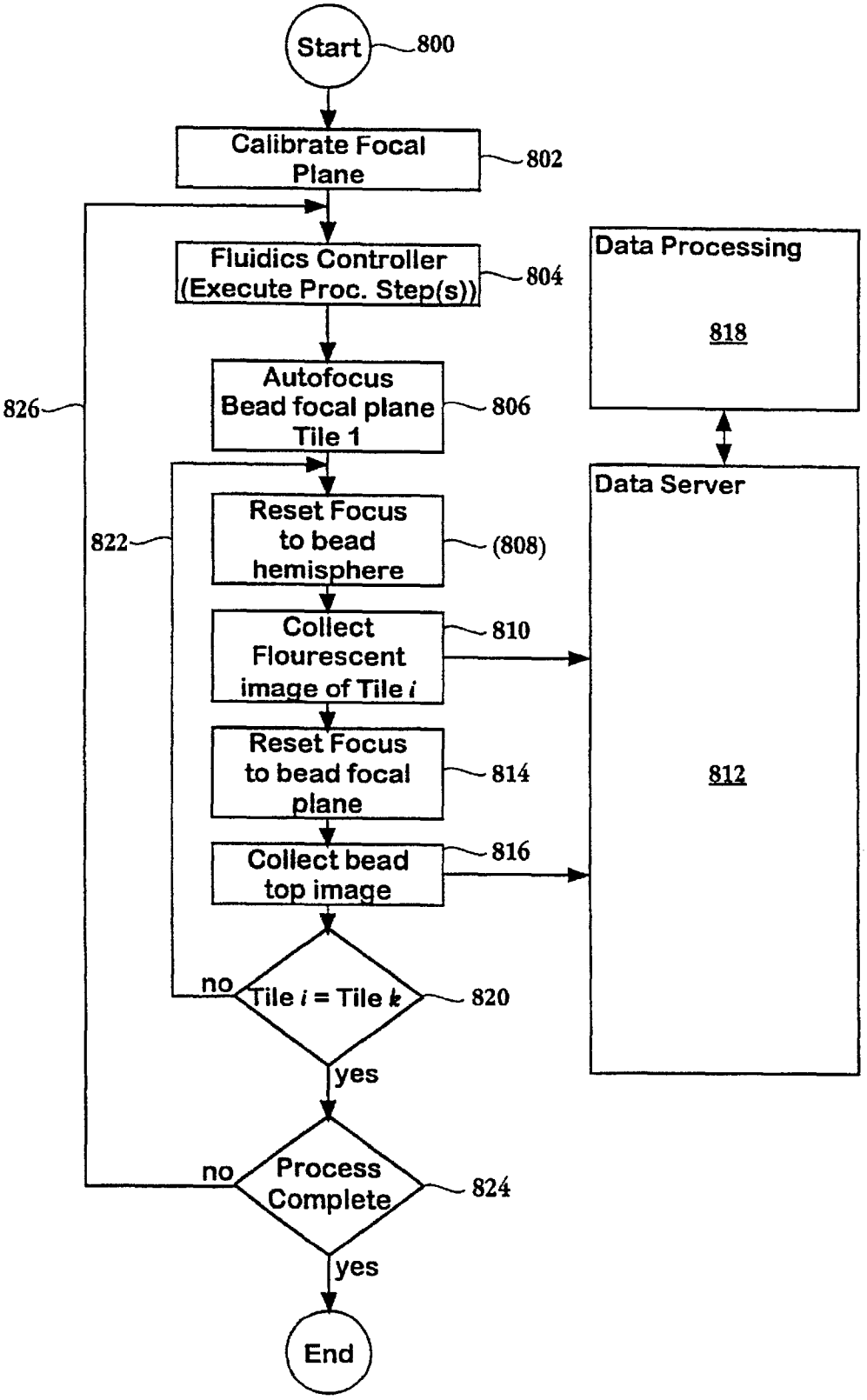


Fig. 7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Calibrate Focal Plane
802
Fluidics Controller (Execute Proc. Step(s))
804
Autofocus Bead focal plane Tile 1
806
826
Reset Focus to bead hemisphere
(808)
822
Collect Flourescent image of Tile i
810
Reset Focus to bead focal plane
814
Collect bead top image
816
Tile i = Tile k
820
no
yes
Process Complete
824
no
yes
End
Data Processing
818
Data Server
812

Fig. 8

SYSTEM AND APPARATUS FOR SEQUENTIAL PROCESSING OF ANALYTES

This application is a divisional of U.S. patent application Ser. No. 09/424,028, filed Nov. 16, 1999, now U.S. Pat. No. 6,406,848, which is a 371 of PCT/US98/11224, filed May 22, 1998, which is a con. of U.S. patent application Serial No. 08/862,610, filed May 23, 1997, now abandoned, all of which are incorporated in their entirety herein by reference.

FIELD OF THE INVENTION

The invention relates generally to systems and apparatus for carrying out large scale parallel reactions on solid phase supports, and more particularly, to systems and apparatus for monitoring and carrying out reactions on arrays of microparticles.

BACKGROUND

The desire to understand and analyze complex chemical and biological systems has led to the development of analytical techniques that employ parallelization and miniaturization of analyte processing, e.g. Graber et al, Current Opinion in Biotechnology, 9: 14–18 (1998); Fodor et al, Nature, 364: 555–556 (1993); Meier-Ewert et al, Nature, 361: 375–376 (1993); Taylor et al, Nucleic Acids Research, 25: 3164–3168 (1997); Garner et al, BioTechniques, 14: 112–115 (1993); Lam et al, Nature, 354: 82–84 (1991); Ohlmeyer et al, Proc. Natl. Acad. Sci., 90: 10922–10926 (1993); DeRisi et al, Science, 278: 680–686 (1997); Wodicka et al, Nature Biotechnology, 15: 1359–1367 (1997); and the like.

Many of these techniques employ microparticles for synthesizing analytes or for capturing analytes for subsequent analysis, e.g. Lam et al (cited above); Benkovic et al, International patent application PCT/US95/03355; Gavin et al, International patent application PCT/EP97/02039; Brenner et al, International patent application PCT/US96/09513, and the like. Even though the properties of different types of microparticles can vary widely, microparticles generally facilitate the construction and manipulation of large repertoires of analytes with minimal reagent and/or sample consumption. However, handling and manipulating large numbers of microparticles, e.g. tens to hundreds of thousands, for carrying out specific chemical and/or biochemical analyses gives rise to many difficulties, including whether sufficient signal is generated on individual microparticles for detection, how to track individual microparticles through multiple steps of a process, mechanical strength of microparticles under pressure or flow conditions, the ability to uniformly deliver reagents to microparticles for carrying out steps of an analytical process, whether clumping or other inappropriate interaction of microparticles and/or reagents occurs, the degree to which analytes and/or processing reagents adsorb onto vessel walls, whether protein reagents or analytes denature causing a disruption of reagent distribution and access, whether adjacent microparticles will interact, e.g. to degrade or obscure a signal or to inhibit reagent access, and the like.

In view of these difficulties, it would be desirable to provide a system and apparatus for handling and processing multiple solid phase supports, such as populations of microparticles. It would be especially desirable if such system and apparatus permitted the tracking and analysis of multiple analytes anchored to separate microparticles through a sequence of several processing and/or analysis steps.

SUMMARY OF THE INVENTION

Accordingly, objects of our invention include, but are not limited to, providing a system and apparatus for sequentially

delivering reagents to a population of analytes anchored to separate microparticles; providing an apparatus for simultaneously monitoring the interactions of processing reagents and analytes on the surfaces of microparticles disposed in a planar array; providing an apparatus for detecting optical signals generated by, or as the result of, interactions of processing reagents and analytes on the surfaces of microparticles disposed in a planar array; providing an apparatus for detecting pluralities of optical signals, each such plurality being generated at the surface of the same microparticle as a result of interactions between processing reagents and an analyte anchored to the surface of such microparticle; providing an apparatus for simultaneously tracking the positions of individual microparticles in a population of microparticles disposed in a flow chamber as a closely packed planar array; and providing a system and apparatus for simultaneously analyzing the nucleotide sequences of a population of polynucleotides anchored to microparticles disposed in a planar array in a flow chamber.

Our invention achieves these and other objects with an apparatus comprising a flow chamber for disposing a population of microparticles in a planar array; fluidic means for sequentially delivering processing reagents from one or more reagent reservoirs to the flow chamber; and detection means for detecting a sequence of optical signals from each of the microparticles of the population. Preferably, the sequences of optical signals are generated as a result of a multi-step analytical process, such as nucleic acid sequence analysis.

In one aspect, the invention provides a system for simultaneously monitoring a population of analytes which includes the apparatus of the invention, microparticles carrying the analytes, and software means for processing images of, and/or optical signals generated by, the microparticles when disposed in a planar array. Preferably, the flow chamber includes constraining means for restricting the movement of microparticles during cycles of reagent delivery.

In another aspect, the invention includes a system for simultaneously analyzing the nucleotide sequences of a population of polynucleotides. Copies of each kind of polynucleotide in the population are sorted onto and anchored to one or more microparticles so that a population of loaded microparticles is formed. Loaded microparticles are disposed in a planar array in a flow chamber through which processing reagents are sequentially delivered to the loaded microparticles from one or more reagent reservoirs by a fluidic means. Optical signals generated by, or produced as a result of, the interaction of processing reagents and polynucleotides on the microparticles are imaged by a detection means. Preferably, when analysis includes determining the nucleotide sequence of a portion of each polynucleotide on the different microparticles, massively parallel signature sequencing (MPSS) analysis is employed, e.g. as described in Albrecht et al, International patent application PCT/US97/09472.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1***a* is a schematic representation of a flow chamber and fluidics and detection systems for observing a planar array of microparticles loaded with analyte molecules, such as cDNA molecules for sequencing.

FIG. **1***b* is a schematic of a preferred holder for a flow chamber.

FIG. **2***a* is bilateral cut away view of a flow chamber.

FIG. **2***b* is a top view of a flow chamber.

FIG. **2***c* is an illustration of microparticles being loaded into a flow chamber.

FIGS. **3***a* through **3***d* schematically illustrate microparticle constraining means for a flow chamber.

FIG. **4** is a schematic representation of a device for loading microparticles into a flow chamber.

FIG. **5** is a schematic representation of a fluidics system for use with the invention.

FIGS. **6***a* and **6***b* schematically illustrate top-lighting and back-lighting approaches for determining microparticle centers in an array.

FIG. **7** schematically illustrates the assignment of pixels to microparticles for data processing.

FIG. **8** is a flow chart summarizing operation of the system of the invention.

DEFINITIONS

"Complement" or "tag complement" as used herein in reference to oligonucleotide tags refers to an oligonucleotide to which a oligonucleotide tag specifically hybridizes to form a perfectly matched duplex or triplex. In embodiments where specific hybridization results in a triplex, the oligonucleotide tag may be selected to be either double stranded or single stranded. Thus, where triplexes are formed, the term "complement" is meant to encompass either a double stranded complement of a single stranded oligonucleotide tag or a single stranded complement of a double stranded oligonucleotide tag.

The term "oligonucleotide" as used herein includes linear oligomers of natural or modified monomers or linkages, including deoxyribonucleosides, ribonucleosides, anomeric forms thereof, peptide nucleic acids (PNAs), and the like, capable of specifically binding to a target polynucleotide by way of a regular pattern of monomer-to-monomer interactions, such as Watson-Crick type of base pairing, base stacking, Hoogsteen or reverse Hoogsteen types of base pairing, or the like. Usually monomers are linked by phosphodiester bonds or analogs thereof to form oligonucleotides ranging in size from a few monomeric units, e.g. 3–4, to several tens of monomeric units, e.g. 40–60. Whenever an oligonucleotide is represented by a sequence of letters, such as "ATGCCTG," it will be understood that the nucleotides are in 5'→3' order from left to right and that "A" denotes deoxyadenosine, "C" denotes deoxycytidine, "G" denotes deoxyguanosine, and "T" denotes thymidine, unless otherwise-noted. Usually oligonucleotides of the invention comprise the four natural nucleotides; however, they may also comprise non-natural nucleotide analogs. It is clear to those skilled in the art when oligonucleotides having natural or non-natural nucleotides may be employed, e.g. where processing by enzymes is called for, usually oligonucleotides consisting of natural nucleotides are required.

"Perfectly matched" in reference to a duplex means that the poly- or oligonucleotide strands making up the duplex form a double stranded structure with one other such that every nucleotide in each strand undergoes Watson-Crick basepairing with a nucleotide in the other strand. The term also comprehends the pairing of nucleoside analogs, such as deoxyinosine, nucleosides with 2-aminopurine bases, and the like, that may be employed. In reference to a triplex, the term means that the triplex consists of a perfectly matched duplex and a third strand in which every nucleotide undergoes Hoogsteen or reverse Hoogsteen association with a basepair of the perfectly matched duplex. Conversely, a "mismatch" in a duplex between a tag and an oligonucleotide means that a pair or triplet of nucleotides in the duplex or triplex fails to undergo Watson-Crick and/or Hoogsteen and/or reverse Hoogsteen bonding.

As used herein, "nucleoside" includes the natural nucleosides, including 2'-deoxy and 2'-hydroxyl forms, e.g. as described in Kornberg and Baker, DNA Replication, 2nd Ed. (Freeman, San Francisco, 1992). "Analogs" in reference to nucleosides includes synthetic nucleosides having modified base moieties and/or modified sugar moieties, e.g. described by Scheit, Nucleotide Analogs (John Wiley, New York, 1980); Uhlman and Peyman, Chemical Reviews, 90: 543–584 (1990), or the like, with the only proviso that they are capable of specific hybridization. Such analogs include synthetic nucleosides designed to enhance binding properties, reduce complexity, increase specificity, and the like.

As used herein "sequence determination" or "determining a nucleotide sequence" in reference to polynucleotides includes determination of partial as well as full sequence information of the polynucleotide. That is, the term includes sequence comparisons, fingerprinting, and like levels of information about a target polynucleotide, as well as the express identification and ordering of nucleosides, usually each nucleoside, in a target polynucleotide. The term also includes the determination of the identification, ordering, and locations of one, two, or three of the four types of nucleotides within a target polynucleotide. For example, in some embodiments sequence determination may be effected by identifying the ordering and locations of a single type of nucleotide, e.g. cytosines, within the target polynucleotide "CATCGC . . . " so that its sequence is represented as a binary code, e.g. "100101 . . . " for "C-(not C)-(not C)-C-(not C)-C . . . " and the like.

As used herein, the term "complexity" in reference to a population of polynucleotides means the number of different species of molecule present in the population.

DETAILED DESCRIPTION OF THE INVENTION

The system and apparatus of the invention is particularly applicable to the analysis of molecules that can be anchored in populations of duplicate copies to particulate solid phase supports. That is, in accordance with the invention, each analyte of a population is present on at least one microparticle in a quantity sufficient for the type of analysis being performed. For example, if combinatorially synthesized peptides on the microparticles are screened against a soluble receptor protein for detecting those that form stable complexes, the number of peptides available for binding on the surface of the microparticles must be large enough to generate a detectable signal when a binding event occurs. Of course, many additional factors well known in the art will present additional design constraints, such as the nature of the system for generating optical signals, the concentration of receptors, pH, salt concentration, the density and accessibility of the peptides on the microparticle surface, the solvent system employed, and the like. Analyte populations particularly relevant for use with the present apparatus include combinatorial libraries synthesized on microparticle supports, e.g as disclosed in Lam et al, Chem. Rev., 97: 411–448 (1997); or Dower et al, U.S. Pat. No. 5,708,153, and polynucleotide libraries sorted onto microparticle supports, e.g. as disclosed in Brenner (cited above).

FIG. **1***a* is a schematic representation of an embodiment of the invention for detecting fluorescent signals. Flow chamber (**100**) having inlet (**102**), outlet (**104**) and planar

cavity (**106**) holds microparticles in a planar array from which optical signals (**108**) generated by analytes and/or reactants on microparticles can be collected and imaged. Flow chamber (**100**) is operationally associated with fluidic system (**112**) and detection system (**114**), so that delivery of fluids and collection of signals is under control of computer (**116**). Preferably, optical signals are collected by microscope (**118**) and are imaged onto a solid state imaging device, such as charge-coupled device (CCD) (**120**) which is capable of generating a digital image of the physical image of the microparticle array with sufficient resolution for individual microparticles to be distinguished. For fluorescent signals, detection system (**114**) usually includes appropriate bandpass filter (**122**) for optical signal (**108**), bandpass filter (**124**) for excitation beam (**128**) generated by light source (**126**), and other standard components. As illustrated, a conventional fluorescence microscope is preferred which is configured for epiillumination. There is a great deal of guidance in the art for selecting appropriate fluorescence microscopes, e.g Wang and Taylor, editors, Fluroescence Microscopy of Living Cells in Culture, Parts A and B, Methods in Cell Biology, Vols. 29 and 30 (Academic Press, New York, 1989).

A key feature of the invention is flow chamber (**100**). Body (**130**) of flow chamber (**100**) preferably comprised inlet (**102**), outlet (**104**) and planar cavity (**106**) which are formed by standard micromachining techniques, e.g. Ekstrom et al, International patent application PCT/SE91/00327; Brown, U.S. Pat. No. 4,911,782; Harrison et al, Anal. Chem. 64: 192–1932 (1992); and the like. Transparent plate (**132**) is sealingly attached to body (**130**) to form an operational flow chamber (**100**). Body (**130**) may be constructed from any of several different materials including glass, silicon, polyethylene, polyester, teflon, other plastics, and the like. Preferably, transparent plate (**132**) is glass or quartz; and, when body (**130**) and transparent plate (**132**) are glass or silicon, transparent plate (**132**) is preferably attached to body (**130**) by anodic bonding, e.g. Pomerantz, U.S. Pat. No. 3,397,279. Key functions of the flow chamber include i) holding a population of microparticles in a substantially immobilized planar array, or monolayer, during a sequence of processing steps, ii) ensuring that processing reagents can access each microparticle during each step of a process, and iii) minimizing processing reagent usage. The degree of immobilization required may vary among different embodiments. Generally, more movement of microparticles within a planar array increases the computational and measurement burden of tracking positions of microparticles by image processing software. Design trade-offs therefore exist between the use of image processing software and the use of physical and/or chemical means for constraining microparticle movement. Preferably, physical and/or chemical means are employed to constrain microparticle movement within the planar array of microparticles in flow chamber (**100**). Such means are referred to herein as "movement constraining means." Most preferably, physical, or mechanical, movement constraining means are employed.

Preferably, microparticles are disposed in flow chamber (**100**) in a closely packed planar array. As used herein, "closely packed" in reference to a planar array means either that the number of microparticles per unit area of a planar array is at least eighty percent of the number of microparticles in a hexagonal array of equal area, or that the average distance between centers of adjacent microparticles is less than two microparticle diameters. As used herein, a "hexagonal" array of microparticles means a planar array of microparticles in which every microparticle in the array contacts at least six other adjacent microparticles, as shown in FIG. **3***a*.

Additions features of flow chamber (**100**) of a preferred embodiment are illustrated in FIGS. **2***a* through **2***c*. FIG. **2***a* is a cross sectional view along a longitudinal plane that bisects flow chamber (**100**). The same view, in a more abstracted rendition, is shown in FIG. **2***c*. In both Figures, inlet (**102**) fluidly communicates with planar cavity (**106**) and outlet (**104**). Microparticles (**200**) carrying analytes enter inlet (**102**) and are carried by a suspending buffer to planar cavity (**106**) where they become packed against dam (**202**) which prevents the microparticles from exiting the flow chamber through outlet (**104**). Structurally, dam (**202**) may be formed by a sudden reduction of the vertical dimension of planar cavity (**106**). Preferably, vertical dimension (**204**) of planar cavity (**106**) is selected so that microparticles (**200**) are constrained to a plane, i.e. a monolayer, when they pack against dam (**202**). More preferably, vertical dimension (**204**) is selected to be between about 120 to 150 percent of the diameter of the microparticles employed. For example, when microparticles are employed that have diameters of 5 μm, vertical dimension (**204**) may be 7 μm. Magnetic microparticles may be constrained to a plane and constrained from movement by applying a magnetic field so that the microparticles are attracted to the ceiling or to the floor of planar cavity (**106**). Width (**206**) of planar cavity (**106**) is not a critical dimension; however, for convenience and efficiency, width (**206**) may be selected to correspond to the dimensions of the signal collection region of detection system (**114**). Such regions labeled l through k in FIG. **2***b* are referred to herein as "tiles." That is, the region of planar cavity (**106**) occupied by microparticles may be divided into non-overlapping areas, referred to as "tiles," that cover the entire occupied region. FIG. **2***b*, which is a top view of the flow chamber of FIG. **2***a*, also shows inlet (**102**), planar cavity (**106**), dam (**202**), and outlet (**104**) that lie in sequence along axis (**217**) of flow chamber (**100**).

Many movement constraining means may be selected for use with the flow chamber, either alone or in combination. Such means include loading microparticles with trace amounts of a chemically reactive species which may be activated and cross-linked; providing physical, or mechanical structures, such as ridges, within the flow chamber; providing magnetically responsive microparticles which may be immobilized by an external magnetic field; providing a second population of microparticles that are loaded into a flow chamber after the analyte-containing population, which forces the analyte-containing population against dam (**202**); and the like. Exemplary chemically reactive species for use with nucleic acid analytes are disclosed in Summerton et al, U.S. Pat. No. 4,123,610; Gamper et al, J. Mol. Biol., 197: 349–362 (1987); Hearst, Ann. Rev. Phys. Chem. 39: 291–315 (1988); Pieles et al, Nucleic Acids Research, 17: 8967–8978 (1989); and the like.

Preferably, microparticle movement is constrained by providing a flow chamber with planar cavity (**106**) containing a plurality of ridges running parallel to axis (**217**) of the flow chamber, i.e. parallel to the direction of reagent flow, so that microparticles are arranged into rows, which may be single-file, or several microparticles wide, as shown in FIGS. **3***a* and **3***b*. The particular selection may depend on several factors, including the degree of immobilization desired, constraints imposed by the fabrication technique used to construct the flow chamber, the amount of reagent access desired, the degree to which flow resistance or back-pressure can be tolerated, and the like. FIGS. **3***a* and **3***b* illustrate two possible distances between parallel ridges. In FIG. **3***a*, the distance is selected to permit maximal packing of microparticles into a hexagonal array, and in FIG. **3***b*, the

distance is selected for less efficient packing, but for increased reagent access to microparticle surfaces. FIGS. **3***c* and **3***d* are axial views of the flow chamber showing the microparticle arrangements of FIGS. **3***a* and **3***b*, respectively.

In some embodiments, such as those employing enzymatic processes, the inner surfaces of flow chamber (**100**) may be passivated, that is, treated to render such surfaces inert and/or non-adsorbing with respect to enzymes. The type of treatment depends on the sensitivity of the enzymes used in the process, and their affinity for the surfaces. Surface treatments include silanization, e.g. with commercially available reagents (Pierce, Rockford, Ill.); and/or adsorption of various blocking polymers, such as poly-a-alanine, polyglycine, polyadenylic acid, polymaleimide, polyvinylpyrrolidone, or the like, e.g. Shofffier et al, Nucleic Acids Research, 24: 375–379 (1996). Preferably, glass inner surfaces of flow chamber (**100**) are covalently coated with a neutral coating, such as allyl methacrylate, using the technique disclosed in Sandoval et al, U.S. Pat. No. 5,326,738, which is incorporated by reference.

FIG. **1***b* illustrates flow chamber (**100**) mounted between holders (**140**) and (**142**) which sealingly connect inlet (**102**) to inlet tubing (**144**) and outlet (**104**) to outlet tubing (**146**), respectively. Preferably, holder (**140**) contains a rotary valve (not shown) operated by actuator (**148**) that shunts fluid flowing through inlet tubing (**144**) to inlet (**102**) or to waste line (**150**). Such a valve minimizes the amount of process reagent from a previous step that must be passed through flow chamber (**100**) prior to the initiation of the next process step. That is, such a rotary valve permits reagent in inlet tubing (**144**) to be shunted to waste and replaced by processing reagent required for the next step in the process being executed. Preferably, for use in DNA analysis, peltier block (**152**) is employed to control temperature in flow chamber (**100**) and the entire assembly including flow chamber (**100**) and peltier block (**152**) is mounted on xyz-stage (**154**) which is under control of computer (**116**).

Preferably, microparticles are loaded into flow chamber (**100**) prior to attachment of holders (**140**) and (**142**) and the initiation of processing steps. FIG. **4** illustrates a microparticle loader for loading microparticles into flow chamber (**100**). Flow chamber (**100**) is mounted between holders (**400**), (**402**), (**404**), and (**406**). Holders (**400**) and (**402**) sealingly clamp onto the inlet end (**101**) of flow chamber (**100**) and holders (**404**) and (**406**) sealingly clamp onto the outlet end (**103**) of flow chamber (**100**) so that inlet tubing (**408**) is in fluid communication with outlet tubing (**410**) when the microparticle loader is assembled. Inlet tubing (**408**) is connected to syringe (**416**) which is used to drive fluid through flow chamber (**100**). Holder (**400**) is constructed to have conical passage (**412**) which narrows to match the diameter of inlet (**102**) of flow chamber (**100**). After assembly of holders (**400**), (**402**), (**404**), and (**406**) a suspension of microparticles is placed in the conical passage after which fitting (**414**) is sealingly connected to holder (**400**). Fluid pressure and flow generated by syringe (**416**) then drives the microparticles into planar cavity (**106**) and against dam (**202**). In a preferred embodiment which employs 5 μm diameter GMA microparticles carrying DNA, approximately 500 thousand microparticles are loaded into flow chamber (**100**) by placing 5 μL of a 100 thousand microparticle/μL solution (TE buffer, pH 8.0, Sambrook et al, Molecular Cloning, Second Edition (Cold Spring Harbor Laboratory, New York, 1989)) in conical passage (**412**), attaching fitting (**414**), and using syringe (**416**) to drive the microparticles through inlet (**102**) and into planar cavity (**106**). After loading, holders (**400**), (**402**), (**404**), and (**406**) are removed from flow chamber (**100**), which is then mounted on the apparatus as shown in FIG. **1***b*.

Preferably, process reagents are delivered to flow chamber (**100**) by the fluidic system illustrated in FIG. **5** which has the capacity to handle many different reagents for complex analytical processes. In the illustrated embodiment, which is used in connection with DNA sequencing, the fluidics system may accommodate up to 38 reagents, including wash buffers, rinses, enzymes, hybridization probes, adaptors, and the like. Preferably, the function of the fluidics system is the sequential metering of selected processing reagents to flow chamber (**100**). Inlet (**102**) of flow chamber (**100**) is sealingly connected to holder (**140**) which contains rotary valve (actuator shown as **148**) (not shown in FIG. **5**). The function of the rotary valve is described above. A variety of means may be employed for moving processing reagents from reservoirs, through tubing, and into flow chamber (**100**), including gravity feed, pressure feed, and pumps, e.g. peristaltic, syringe, and the like. Preferably, common syringe pump (**500**) is employed for removing predetermined amounts processing reagents from reservoirs and for forcing such reagents through flow chamber (**100**) at a predetermined flow rate. Under control of computer (**116**), pump (**500**) in operational association with valve block (**502**) and rotary valve (**504**) removes a predetermined amount of processing reagent from a selected reservoir by siphoning reagent out of the reservoir on the out-stroke of plunger (**501**) of pump (**500**). On the in-stroke of plunger (**501**), rotary valve (**504**) directs processing reagent from tubing (**503**) to reservoir (**505**) of pump (**500**). On the out-stroke of plunger (**501**), state of rotary valve (**504**) is changed to direct processing reagent from reservoir (**505**) to inlet tubing (**144**). Tubing (**503**) connects rotary valve (**504**) with manifold (**508**) which, in turn, is connected to a plurality (five shown) of banks of zero dead volume valves (**506**). Zero dead volume valves (**506**) connect individual reservoirs holding processing reagents to a common passageway (not shown in FIG. **5**) that runs through each of the banks of valves connecting to manifold (**508**).

A preferred zero dead volume valve is described in U.S. Pat. Nos. 4,558,845 and 4,703,913, which are incorporated by reference. Process reagents from reservoirs (**514**) are distributed to the banks of dead volume valves by way of manifold (**510**). Alternative valve blocks for controlling delivery of process reagents to flow chamber (**100**) include the valve matrix disclosed in U.S. Pat. No. 5,203,368.

An important feature of detection means (**114**) of the invention is the ability to keep track of individual microparticles through multiple process steps and/or cycles. In connection with such tracking, detection means (**114**) periodically records optical characteristics of individual microparticles that provide a close approximation microparticle centers. Preferably, when transillumination, or "back lighting" of flow chamber (**100**) is possible, the optical characteristic is the focused back light from the microparticles. That is, in reference to FIG. **6***a*, back light (**600**) passes vertically through flow chamber (**100**) where it is focused by microparticles (**602**) onto focal plane (**604**). The image of focal plane (**604**) in this configuration appears as a field of bright points, where each point is located at the approximate center of its corresponding microparticle. In an epiillumination system, light from above flow chamber (**100**), i.e. "top light (**610**)," is directed from a vertical direction onto microparticles (**602**) where it scatters from the top surface of the microparticles. In this configuration, the optical characteristic is the scatter center of a microparticle. Thus, an

image is collected from the plane containing scatter centers (612) resulting from such top lighting. As with focused back lighting, the image of the scatter centers provides a convenient way to readily determine the approximate centers of the microparticles.

In the preferred image processing approach, once microparticle centers (700) are determined, pixels (702) are assigned for determining characteristics, e.g. intensity, of an optical signal generated at each microparticle (602). The size of microparticle (602) and pixel area determine how many pixels are assigned to each microparticle. In making such an assignment, important factors include the degree to which the calculated center of a microparticle (as described above) is likely to deviate from the geometric center, the extent to which optical signal collected from the edge of an image contains spurious information (e.g. signal from an overlapping or adjacent microparticle), the uniformity of microparticle diameter and shape, and the like. In the preferred apparatus of the invention, 5 μm diameter microparticles are employed and the pixel dimensions of the CCD detector are about 0.9 μm×0.9 μm. Thus, nine pixels fit easily within the interior of a microparticle image with a margin of at least about 1 μm between any pixel and the edge of the microparticle image. In the preferred embodiment, an initial pixel is assigned which encloses the computed center of a microparticle, e.g. pixel "5" in FIG. 7. Thereafter, additional pixels are assigned, usually the immediately adjacent pixels. Preferably, the value of the optical signal generated by a process at the surface of a microparticle is the average value of the optical signals collected by pixels assigned to that microparticle.

The general operation of the system of the preferred embodiment is summarized by the flow chart of FIG. 8. At the start (800) of an analysis, microparticles with anchored analytes have been loaded into flow chamber (100) which has been operationally mounted in holders 140 and 142. The initial operation is the calibration of the microparticle focal plane (802). That is, the vertical, or "z", position of the xyz-stage is determined which optimizes the focus of either the scatter centers of the microparticles, i.e. the microparticle tops for top-lighting, or the focus points of the microparticles for back-lighting. The optimization is carried out by a conventional autofocusing algorithm which provides an image contrast function constructed from a predetermined sample of regions within a collected image. For example, the contrast function may be evaluated iteratively for sequence of z-positions so that the differences of successive values of the contrast function can be determined. These are tested until a difference is found below a predetermined threshold, which is taken as the maximum of the contrast function. Focal plane location is taken as the z position which maximizes the image contrast function. Such calibration is carried out for each tile, if more than one tile is employed, so that a correction table is constructed of changes in stage setting values with respect to the settings of the first tile that are required to bring the system into focus upon translation to subsequent tiles. These values are stored by computer (116).

After calibration, process steps are initiated (804) by way of a fluidics controller operationally associated with computer (116). After process steps (804) are completed, stage settings are adjusted to place the first tile into focus using the autofocus algorithm (806), which places the focal plane of the microscope objective approximately at the tops of the microparticles. Stage settings are then adjusted (808) to bring the focal plane of the microscope objective to the approximate centers of the microparticles, as illustrated (606) in FIGS. 6*a* and 6*b*. The amount of stage movement in this re-focusing depends on the diameter of the microparticles being used. After appropriate selection of filters (124) and (122), a fluorescent image of the first tile is collected (810) and transferred to data server (812). Fluorescent images are collected on the plane of the microparticle centers because of imperfections in the planar array. That is, microparticles in planar cavity (106) do not lie in a perfect planar array for a variety of reasons. For example, some microparticles are elevated above others as a result of packing into the flow chamber; there is some variability in the size and shape of the microparticles; and, the floor of planar cavity (106) may be uneven. After the fluorescent image is collected, the focal plane of the microscope objective is returned (814) to the microparticle focal plane, where another image is collected (816) for the purpose of computing microparticle centers as described above. The image of microparticle centers is transferred to data server (812) where data processor (818) assigns pixels of the fluorescent image to each microparticle center, as described above. After the image of microparticle centers is collected (816), the stage is moved so that an image of the next tile can be collected (822). If there are no further tiles of microparticles (820), then the next steps and/or cycles of the process are executed (826). If there are no further process steps (824), then the process is complete and the apparatus is placed in a holding mode.

Optical signals collected in the course of analysis may be generated by a variety of mechanisms, including absorption and fluorescence, chemiluminescence, electrochemiluminescence, or bioluminescence emission. Extensive guidance is available for selecting appropriate optical signaling means, e.g. Kessler, editor, Nonradioactive Labeling and Detection of Biomolecules (Springer-Verlag, Berlin); Keller and Manak, DNA Probes, Second Edition (Stockton Press, New York, 1993); and the like. Preferably, optical signals generated in processing steps are fluorescence emissions.

Microparticles

An important feature of the system of the invention is the use of microparticles for carrying analytes. A variety of microparticles may be employed depending on particular applications. Generally, microparticles must consist of a material compatible with the reagents and chemistry of the process steps being carried out and microparticle must be substantially mechanically rigid so that they retain their shape and size during process steps. Preferably, as used herein, the term "substantially mechanically rigid" means that microparticles neither swell nor contract by more than ten percent (as measure by diameter) in any process solvent or reagent. Preferably, microparticles are microspheres of uniform size, i.e. microparticles are monodisperse. More preferably, the diameters of spherical microparticles have a coefficient of variation less than five percent, and most preferably, less than two percent. Microparticle diameters are in the range of from 0.1 μm to 100 μm. Preferably, microparticle diameters range from 1 μm to 20 μm. Most preferably, microparticle diameters are in the range of 1 to 5 μm. Suitable microparticle materials include inorganic support materials such as glass, e.g. controlled-pore glass, Balltoni beads; silica, zirconia, and the like, e.g. Weetall, Methods in Enzymology, 44: 134–148 (1976); and organic support materials such as highly cross-linked polystyrene, polyacrylate, polymethylmethacrylate, glycidylmethacrylate (GMA), Dynabeads (Dynal, Oslo, Norway), and the like, Rembaum et al, U.S. Pat. No. 4,046,720; Hodge and

Sherrington, editors, pages 435–456, Polymer-supported Reactions in Organic Synthesis (Wiley & Sons, New York, 1980); Andrus et al, U.S. Pat. No. 5,047,524; and the like.

Attaching Identical Copies of Polynucleotides to Microparticles by Solid Phase Cloning

In a preferred embodiment of the invention, identical copies of polynucleotides from a population are anchored to separate microparticles by solid phase cloning, i.e. the use of oligonucleotide tags for sorting polynucleotides onto microparticles such that only the same kind of polynucleotide will be attached to the same microparticle, e.g. Brenner, U.S. Pat. No. 5,604,097, which is incorporated by reference. This condition is accomplished by taking a sample of the full ensemble of tag-polynucleotide conjugates. (It is acceptable that identical polynucleotides have different tags, as it merely results in the same polynucleotide being operated on or analyzed twice in two different locations.) Such sampling can be carried out either overtly—for example, by taking a small volume from a larger mixture—after the tags have been attached to the polynucleotides, it can be carried out inherently as a secondary effect of the techniques used to process the polynucleotides and tags, or sampling can be carried out both overtly and as an inherent part of processing steps.

Oligonucleotide tags for use with the invention are members of a minimally cross-hybridizing set of oligonucleotides. The sequences of oligonucleotides of such a set differ from the sequences of every other member of the same set by at least two nucleotides. Thus, each member of such a set cannot form a duplex (or triplex) with the complement of any other member with less than two mismatches. Complements of oligonucleotide tags of the invention, referred to herein as "tag complements," may comprise natural nucleotides or non-natural nucleotide analogs. Tag complements are attached to microparticles.

Minimally cross-hybridizing sets of oligonucleotide tags and tag complements may be synthesized either combinatorially or individually depending on the size of the set desired and the degree to which cross-hybridization is sought to be minimized (or stated another way, the degree to which specificity is sought to be enhanced). For example, a minimally cross-hybridizing set may consist of a set of individually synthesized 10-mer sequences that differ from each other by at least 4 nucleotides, such set having a maximum size of 332 (when composed of 3 kinds of nucleotides and counted using a computer program such as disclosed in Appendix Ic of International patent application PCT/US96/09513). Alternatively, a minimally cross-hybridizing set of oligonucleotide tags may also be assembled combinatorially from subunits which themselves are selected from a minimally cross-hybridizing set. For example, a set of minimally cross-hybridizing 12-mers differing from one another by at least three nucleotides may be synthesized by assembling 3 subunits selected from a set of minimally cross-hybridizing 4-mers that each differ from one another by three nucleotides. Such an embodiment gives a maximally sized set of 9^3, or 729, 12-mers, "9" is number of oligonucleotides generated by the computer program of Appendix Ia of International patent application PCT/US96/09513, which assumes, as with the 10-mers, that only 3 of the 4 different types of nucleotides are used. The set is described as "maximal" because the computer programs disclosed in International patent application PCT/US96/09513 provide the largest set for a given input (e.g. length, composition, difference in number of nucleotides between members). Additional minimally cross-hybridizing sets may be formed from subsets of such calculated sets.

When synthesized combinatorially, an oligonucleotide tag of the invention preferably consists of a plurality of subunits, each subunit consisting of an oligonucleotide of 3 to 9 nucleotides in length wherein each subunit is selected from the same minimally cross-hybridizing set. In such embodiments, the number of oligonucleotide tags available depends on the number of subunits per tag and on the length of the subunits.

As used herein in reference to oligonucleotide tags and tag complements, the term "repertoire" means the set of minimally cross-hybridizing set of oligonucleotides that make up the tags in a particular embodiment or the corresponding set of tag complements.

Preferably, in constructing a cDNA library where substantially all different cDNAs have different tags, a tag repertoire is employed whose complexity, or number of distinct tags, greatly exceeds the total number of mRNAs extracted from a cell or tissue sample. Preferably, the complexity of the tag repertoire is at least 10 times that of the polynucleotide population; and more preferably, the complexity of the tag repertoire is at least 10 times that of the polynucleotide population. Below, a protocol is disclosed for cDNA library construction using a primer mixture that contains a full repertoire of exemplary 9-word tags. Such a mixture of tag-containing primers has a complexity of 8^9, or about 1.34×10^8. As indicated by Winslow et al, Nucleic Acids Research, 19: 3251–3253 (1991), mRNA for library construction can be extracted from as few as 10–100 mammalian cells. Since a single mammalian cell contains about 5×10^5 copies of mRNA molecules of about 3.4×10^4 different kinds, by standard techniques one can isolate the mRNA from about 100 cells, or (theoretically) about 5×10^7 mRNA molecules. Comparing this number to the complexity of the primer mixture shows that without any additional steps, and even assuming that mRNAs are converted into cDNAs with perfect efficiency (1% efficiency or less is more accurate), the cDNA library construction protocol results in a population containing no more than 37% of the total number of different tags. That is, without any overt sampling step at all, the protocol inherently generates a sample that comprises 37%, or less, of the tag repertoire. The probability of obtaining a double under these conditions is about 5%, which is within the preferred range. With mRNA from 10 cells, the fraction of the tag repertoire sampled is reduced to only 3.7%, even assuming that all the processing steps take place at 100% efficiency. In fact, the efficiencies of the processing steps for constructing cDNA libraries are very low, a "rule of thumb" being that good library should contain about 10^8 cDNA clones from mRNA extracted from 10^6 mammalian cells.

Use of larger amounts of mRNA in the above protocol, or for larger amounts of polynucleotides in general, where the number of such molecules exceeds the complexity of the tag repertoire, a tag-polynucleotide conjugate mixture potentially contains every possible pairing of tags and types of mRNA or polynucleotide. In such cases, overt sampling may be implemented by removing a sample volume after a serial dilution of the starting mixture of tag-polynucleotide conjugates. The amount of dilution required depends on the amount of starting material and the efficiencies of the processing steps, which are readily estimated.

If mRNA were extracted from 10^6 cells (which would correspond to about 0.5 μg of poly(A)$^+$ RNA), and if primers were present in about 10–100 fold concentration excess—as is called for in a typical protocol, e.g. Sambrook et al, Molecular Cloning, Second Edition, page 8.61 [10 μL 1.8 kb mRNA at 1 mg/mL equals about 1.68×10^{-11} moles and 10

μL 18-mer primer at 1 mg/mL equals about 1.68×10^{-9} moles], then the total number of tag-polynucleotide conjugates in a cDNA library would simply be equal to or less than the starting number of mRNAs, or about 5×10^{11} vectors containing tag-polynucleotide conjugates—again this assumes that each step in cDNA construction—first strand synthesis, second strand synthesis, ligation into a vector—occurs with perfect efficiency, which is a very conservative estimate. The actual number is significantly less.

If a sample of n tag-polynucleotide conjugates are randomly drawn from a reaction mixture—as could be effected by taking a sample volume, the probability of drawing conjugates having the same tag is described by the Poisson distribution, $P(r)=e^{-\lambda}(\lambda)^r/r$, where r is the number of conjugates having the same tag and $\lambda=np$, where p is the probability of a given tag being selected. If $n=10^6$ and $p=1/(1.34\times10^8)$,then $\lambda=0.00746$ and $P(2)=2.7\times10^{-5}$. Thus, a sample of one million molecules gives rise to an expected number of doubles well within the preferred range. Such a sample is readily obtained as follows: Assume that the 5×10^{11} mRNAs are perfectly converted into 5×10^{11} vectors with tag-cDNA conjugates as inserts and that the 5×10^{11} vectors are in a reaction solution having a volume of 100 μl. Four 10-fold serial dilutions may be carried out by transferring 10 μl from the original solution into a vessel containing 90 μl of an appropriate buffer, such as TE. This process may be repeated for three additional dilutions to obtain a 100 μl solution containing 5×10^5 vector molecules per μl. A 2 μl aliquot from this solution yields 10^6 vectors containing tag-cDNA conjugates as inserts. This sample is then amplified by straight forward transformation of a competent host cell followed by culturing.

Of course, as mentioned above, no step in the above process proceeds with perfect efficiency. In particular, when vectors are employed to amplify a sample of tag-polynucleotide conjugates, the step of transforming a host is very inefficient. Usually, no more than 1% of the vectors are taken up by the host and replicated. Thus, for such a method of amplification, even fewer dilutions would be required to obtain a sample of 10^6 conjugates.

A repertoire of oligonucleotide tags can be conjugated to a population of polynucleotides in a number of ways, including direct enzymatic ligation, amplification, e.g. via PCR, using primers containing the tag sequences, and the like. The initial ligating step produces a very large population of tag-polynucleotide conjugates such that a single tag is generally attached to many different polynucleotides. However, as noted above, by taking a sufficiently small sample of the conjugates, the probability of obtaining "doubles," i.e. the same tag on two different polynucleotides, can be made negligible. Generally, the larger the sample the greater the probability of obtaining a double. Thus, a design trade-off exists between selecting a large sample of tag-polynucleotide conjugates—which, for example, ensures adequate coverage of a target polynucleotide in a shotgun sequencing operation or adequate representation of a rapidly changing mRNA pool, and selecting a small sample which ensures that a minimal number of doubles will be present. In most embodiments, the presence of doubles merely adds an additional source of noise or, in the case of sequencing, a minor complication in scanning and signal processing, as microparticles giving multiple fluorescent signals can simply be ignored.

As used herein, the term "substantially all" in reference to attaching tags to molecules, especially polynucleotides, is meant to reflect the statistical nature of the sampling procedure employed to obtain a population of tag-molecule conjugates essentially free of doubles. The meaning of substantially all in terms of actual percentages of tag-molecule conjugates depends on how the tags are being employed. Preferably, for nucleic acid sequencing, substantially all means that at least eighty percent of the polynucleotides have unique tags attached. More preferably, it means that at least ninety percent of the polynucleotides have unique tags attached. Still more preferably, it means that at least ninety-five percent of the polynucleotides have unique tags attached. And, most preferably, it means that at least ninety-nine percent of the polynucleotides have unique tags attached.

Tags can be conjugated to cDNAs of existing libraries by standard cloning methods. cDNAs are excised from their existing vector, isolated, and then ligated into a vector containing a repertoire of tags. Preferably, the tag-containing vector is linearized by cleaving with two restriction enzymes so that the excised cDNAs can be ligated in a predetermined orientation. The concentration of the linearized tag-containing vector is in substantial excess over that of the cDNA inserts so that ligation provides an inherent sampling of tags.

A general method for exposing the single stranded tag after amplification involves digesting a target polynucleotide-containing conjugate with the 5'3' exonuclease activity of T4 DNA polymerase, or a like enzyme, e.g. as described in Kuijper et al, Gene, 112: 147–155 (1992). When used in the presence of a single deoxynucleoside triphosphate, such a polymerase will cleave nucleotides from 3' recessed ends present on the non-template strand of a double stranded fragment until a complement of the single deoxynucleoside triphosphate is reached on the template strand. When such a nucleotide is reached the 5'→3' digestion effectively ceases, as the polymerase's extension activity adds nucleotides at a higher rate than the excision activity removes nucleotides. Consequently, single stranded tags constructed with three nucleotides are readily prepared for loading onto solid phase supports.

After the oligonucleotide tags are prepared for specific hybridization, e.g. by rendering them single stranded as described above, the polynucleotides are mixed with microparticles containing the complementary sequences of the tags under conditions that favor the formation of perfectly matched duplexes between the tags and their complements. There is extensive guidance in the literature for creating these conditions. Exemplary references providing such guidance include Wetmur, Critical Reviews in Biochemistry and Molecular Biology, 26: 227–259 (1991); Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd Edition (Cold Spring Harbor Laboratory, New York, 1989); and the like. Preferably, the hybridization conditions are sufficiently stringent so that only perfectly matched sequences form stable duplexes. Under such conditions the polynucleotides specifically hybridized through their tags may be ligated to the complementary sequences attached to the microparticles. Finally, the microparticles are washed to remove polynucleotides with unligated and/or mismatched tags.

Preferably, for sequencing applications, standard CPG beads of diameter in the range of 20–50 μm are loaded with about 10^5 polynucleotides, and glycidalmethacrylate (GMA) beads available from Bangs Laboratories (Carmel, Ind.) of diameter in the range of 5–10 μm are loaded with a few tens of thousand polynucleotide, e.g. 4×10^4 to 6×10^4, to a hundred thousand polynucleotides.

DNA Sequencing

Polynucleotides loaded onto microparticles may be simultaneously sequenced in the instant apparatus using a "base-by-base" DNA sequencing methodology. Such sequencing methodology permits the stepwise identification of a sequence of nucleotides in a target polynucleotide, usually one base at a time, through successive cycles of treatment and detection. Base-by-base approaches are disclosed in the following references: Cheeseman, U.S. Pat. No. 5,302,509; Tsien et al, International application WO 91/06678; Rosenthal et al, International application WO 93/21340; Canard et al, Gene, 148: 1–6 (1994); Metzker et al, Nucleic Acids Research, 22: 4259–4267 (1994); and the like. Preferably, the base-by-base approach disclosed by Brenner in U.S. Pat. No. 5,599,675 is used with the apparatus of the invention to sequence polynucleotides on a population of loaded microparticles disposed as a planar array in the flow chamber. Accordingly, Brenner, U.S. Pat. No. 5,599,675 is incorporated by reference. Preferably, the a population of loaded microparticles for sequencing includes at least ten thousand loaded microparticles; more preferably, such a population includes at least fifty thousand loaded microparticles; and still more preferably, such a population includes at least one hundred thousand loaded microparticles.

Preferably, the sequencing method of Brenner (cited above) is employed in the embodiment disclosed in Albrecht et al International patent application PCT/US97/109472 which discloses the use of encoded adaptors. An encoded adaptor is a double stranded oligonucleotide comprising a protruding strand and an oligonucleotide tag selected from a minimally cross-hybridizing set of oligonucleotides. Encoded adaptors whose protruding strands form perfectly matched duplexes with the complementary protruding strands of the target polynucleotide are ligated. After ligation, the identity and ordering of the nucleotides in the protruding strands are determined, or "decoded," by specifically hybridizing a labeled tag complement to its corresponding tag on the ligated adaptor. Encoded adaptors may be used in an adaptor-based method of DNA sequencing that includes repeated cycles of ligation, identification, and cleavage, such as the method described in Brenner (cited above). Briefly, such a method comprises the following steps: (a) ligating an encoded adaptor to an end of a polynucleotide, the encoded adaptor having a nuclease recognition site of a nuclease whose cleavage site is separate from its recognition site; (b) identifying one or more nucleotides at the end of the polynucleotide by the identity of the encoded adaptor ligated thereto; (c) cleaving the polynucleotide with a nuclease recognizing the nuclease recognition site of the encoded adaptor such that the polynucleotide is shortened by one or more nucleotides; and (d) repeating said steps (a) through (c) until said nucleotide sequence of the polynucleotide is determined. In the identification step, successive sets of tag complements are specifically hybridized to the respective tags carried by encoded adaptors ligated to the ends of the target polynucleotides, as described above. The type and sequence of nucleotides in the protruding strands of the polynucleotides are identified by the label carried by the specifically hybridized tag complement and the set from which the tag complement came.

Construction and Sorting of cDNA Library for Signature Sequencing with Encoded Adaptors

In this example, a cDNA library is constructed in which an oligonucleotide tag consisting of 8 four-nucleotide "words" is attached to each cDNA. As described above, the repertoire of oligonucleotide tags of this size is sufficiently large (about 10^8) so that if the cDNAs are synthesized from a population of about 10^6 mRNAs, then there is a high probability that each cDNA will have a unique tag for sorting. After mRNA extraction, first strand synthesis is carried out in the presence of 5-Me-dCTP (to block certain cDNA restriction sites) and a biotinylated primer mixture containing the oligonucleotide tags. After conventional second strand synthesis, the tag-cDNA conjugates are cleaved with Dpn II (which is unaffected by the 5-Me-deoxycytosines), the biotinylated portions are separated from the reaction mixture using streptavidin-coated magnetic beads, and the tag-cDNA conjugates are recovered by cleaving them from the magnetic beads via a Bsm BI site carried by the biotinylated primer. The Bsm BI-Dpn II fragment containing the tag-cDNA conjugate is then inserted into a plasmid and amplified. After isolation of the plasmids, tag-cDNA conjugates are amplified out of the plasmids by PCR in the presence of 5-Me-dCTP, using biotinylated and fluorescently labeled primers containing pre-defined restriction endonuclease sites. After affinity purification with streptavidin coated magnetic beads, the tag-cDNA conjugates are cleaved from the beads, treated with T4 DNA polymerase in the presence of dGTP to render the tags single stranded, and then combined with a repertoire of GMA beads having tag complements attached. After stringent hybridization and ligation, the GMA beads are sorted via FACS to produce an enriched population of GMA beads loaded with cDNAs. The enriched population of loaded GMA beads are immobilized in a planar array in a flow chamber where base-by-base sequence takes place using encoded adaptors, as disclosed in Albrecht et al, International patent application PCT/US97/09472.

Approximately 5 μg of poly(A^+) mRNA is extracted from DBY746 yeast cells using conventional protocols. First and second strand cDNA synthesis is carried out by combining 100–150 pmoles of the following primer (SEQ ID NO: 1):

5'-biotin-
ACTAATCGTCTCACTATTTAATTAA[W,W,W,G]$_8$CC(T)$_{18}$V-3'

with the poly(A^+) mRNA using a Stratagene (La Jolla, Calif.) cDNA Synthesis Kit in accordance with the manufacturer's protocol. This results in cDNAs whose first stand deoxycytosines are methylated at the 5-carbon position. In the above formula, "V" is G, C, or A, "[W,W,W,G]" is a four-nucleotide word selected from Table II of Brenner, International patent application PCT/US96/09513, the single underlined portion is a Bsm BI recognition site, and the double underlined portion is a Pac I recognition site. After size fractionation (GIBCO-BRL cDNA Size Fractionation Kit) using conventional protocols, the cDNAs are digested with Dpn II (New England Bioscience, Beverly, Mass.) using manufacturer's protocol and affinity purified with streptavidin-coated magnetic beads (M-280 beads, Dynal A. S., Oslo, Norway). The DNA captured by the beads is digested with Bsm BI to release the tag-cDNA conjugates for cloning into a modified pBCSK$^-$ vector (Stratagene, La Jolla, Calif.) using standard protocols. The pBCSK$^-$ vector is modified by adding a Bbs I site by inserting the following fragment (SEQ ID NO: 2) into the Kpn I/Eco RV digested vector.

CGAAGACCC
3'-CATGGCTTCTGGGGATA-5'

Bsm BI/Dpn II digested tag-cDNA conjugate is inserted in the pBCSK⁻ which is previously digested with Bbs I and Bam HI. After ligation, the vector is transfected into the manufacturer's recommended host for amplification.

After isolating the above pBCSK⁻ vector from a standard plasmid miniprep, the tag-cDNA conjugates are amplified by PCR in the presence of 5-Me-dCTP using 20-mer primers complementary to vector sequences flanking the tag-cDNA insert. The "upstream" primer, i.e. adjacent to the tag, is biotinylated and the "downstream" primer, i.e. adjacent to the cDNA, is labeled with fluorescein. After amplification, the PCR product is affinity purified then cleaved with Pac I to release fluorescently labeled tag-cDNA conjugates. The tags of the conjugates are rendered single stranded by treating them with T4 DNA polymerase in the presence of dGTP. After the reaction is quenched, the tag-cDNA conjugate is purified by phenol-chloroform extraction and combined with 5.5 mm GMA beads carrying tag complements, each tag complement having a 5' phosphate. Hybridization is conducted under stringent conditions in the presence of a thermal stable ligase so that only tags forming perfectly matched duplexes with their complements are ligated. The GMA beads are washed and the loaded beads are concentrated by FACS sorting, using the fluorescently labeled cDNAs to identify loaded GMA beads. The tag-cDNA conjugates attached to the GMA beads are digested with Dpn II to remove the fluorescent label and treated with alkaline phosphatase to prepare the cDNAs for sequencing. That is, phasphatase is used to remove the 5' phosphate from the ends of the cDNAs to prevent unwanted cDNA-cDNA ligations by way of the palindromic Dpn II site.

The following cleavage adaptor (SEQ ID NO: 3) is ligated to the Dpn II-digested and phosphatase treated cDNAs:

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5'-pGATCAGCTGCTGCAAATTT
      pTCGACGACGTTTAAA
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After ligation, the 3' phosphate is removed by alkaline phosphatase, the 5' strand of the cDNA is treated with T4 DNA kinase, and the nick between the cleavage adaptor and cDNA is ligated. After cleavage by Bbv I, encoded adaptors are ligated to the ends of the cDNAs and the beads are ready for loading into the flow chamber.

Ligation of the adaptors to the target polynucleotide is carried out in a mixture consisting of 5 μl beads (20 mg), 3 μL NEB 10× ligase buffer, 5 μL adaptor mix (25 nM), 2.5 μL NEB T4 DNA ligase (2000 units/μL), and 14.5 μL distilled water. The mixture is incubated at 16° C. for 30 minutes, after which the beads are washed 3 times in TE (pH 8.0).

After centrifugation and removal of TE, the 3' phosphates of the ligated adaptors are removed by treating the polynucleotide-bead mixture with calf intestinal alkaline phosphatase (CIP) (New England Biolabs, Beverly, Mass.), using the manufacturer's protocol. After removal of the 3' phosphates, the CIP may be inactivated by proteolytic digestion, e.g. using Pronase™ (available form Boeringer Mannhiem, Indianapolis, Ind.), or an equivalent protease, with the manufacturer's protocol. The polynucleotide-bead mixture is then washed, treated with a mixture of T4 polynucleotide kinase and T4 DNA ligase (New England Biolabs, Beverly, Mass.) to add a 5' phosphate at the gap between the target polynucleotide and the adaptor, and to complete the ligation of the adaptors to the target polynucleotide. The bead-polynucleotide mixture is then washed in TE, diluted to a concentration of approximately 100 thousand beads per μL, and 5 μL of the resulting solution is loaded into a flow chamber with the help of the holders of FIG. **4**.

The top strands of the following 16 sets of 64 encoded adaptors (SEQ ID NO: 4 through SEQ ID NO: 19) are each separately synthesized on an automated DNA synthesizer (model 392 Applied Biosystems, Foster City) using standard methods. The bottom strand, which is the same for all adaptors, is synthesized separately then hybridized to the respective top strands:

SEQ ID NO.	Encoded Adaptor
4	5'-pANNNTACAGCTGCATCCCttggcgctgagg pATGCACGCGTAGGG-5'
5	5'-pNANNTACAGCTGCATCCCtgggcctgtaag pATGCACGCGTAGGG-5'
6	5'-pCNNNTACAGCTGCATCCCttgacgggtctc pATGCACGCGTAGGG-5'
7	5'-pNCNNTACAGCTGCATCCCtgcccgcacagt pATGCACGCGTAGGG-5'
8	5'-pGNNNTACAGCTGCATCCCttcgcctcggac pATGCACGCGTAGGG-5'
9	5'-pNGNNTACAGCTGCATCCCtgatccgctagc pATGCACGCGTAGGG-5'
10	5'-pTNNNTACAGCTGCATCCCttccgaacccgc pATGCACGCGTAGGG-5'
11	5'-pNTNNTACAGCTGCATCCCtgaggggatag pATGCACGCGTAGGG-5'
12	5'-pNNANTACAGCTGCATCCCttcccgctacac pATGCACGCGTAGGG-5'
13	5'-pNNNATACAGCTCCATCCCtgactccccgag pATGCACGCGTAGGG-5'
14	5'-pNNCNTACAGCTGCATCCCtgtgttgcgcgg pATGCACGCGTAGGG-5'
15	5'-pNNNCTACAGCTGCATCCCtctacagcagcg pATGCACGCGTAGGG-5'
16	5'-pNNGNTACAGCTGCATCCCtgtcgcgtcgtt pATGCACGCGTAGGG-5'
17	5'-pNNNGTACAGCTGCATCCCtcggagcaacct pATGCACGCGTAGGG-5'
18	5'-pNNTNTACAGCTGCATCCCtggtgaccgtag pATGCACGCGTAGGG-5'
19	5'-pNNNTTACAGCTGCATCCCtcccctgtcgga pATGCACGCGTAGGG-5'

where N and p are as defined above, and the nucleotides indicated in lower case letters are the 12-mer oligonucleotide tags. Each tag differs from every other by 6 nucle-

otides. Equal molar quantities of each adaptor are combined in NEB #2 restriction buffer (New England Biolabs, Beverly, Mass.) to form a mixture at a concentration of 1000 pmol/μL.

Each of the 16 tag complements are separately synthesized as amino-derivatized oligonucleotides and are each labeled with a fluorescein molecule (using an NHS-ester of fluorescein, available from Molecular Probes, Eugene, Oreg.) which is attached to the 5' end of the tag complement through a polyethylene glycol linker (Clonetech Laboratories, Palo Alto, Calif.). The sequences of the tag complements are simply the 12-mer complements of the tags listed above.

A flow chamber of the design shown in FIGS. **2***a* and **2***b* is employed in association with an Olympus Optical Co., Ltd. (Tokyo, Japan) model BX60MF5 fluorescent microscope fitted with a model U-ULS75XE 75 watt Xenon arc lamp, a motorized filter wheel, a Ludl Electronic Products, Ltd. computer-controlled stage, and a Photometrics, Ltd. (Tucson, Ariz.) PXL CCD camera with a 2000×2000 pixel array. Appropriate bandpass filters (**122**) and (**124**) are employed for exciting fluorescein and transmitting fluorescent signal to CCD camera (**120**). Microparticle positions are determined by top-lighting with broadband light from Xenon lamp (**126**) reduced by a factor of about 10^{-4} with a neutral density filter. Fluorescent images are collected with about 2 minute exposure times.

Height (**204**) of flow chamber (**201**) is selected to be 7 μm, or approximately 140% of the diameter of the GMA beads. Width (**210**) of flow chamber (**201**) is selected so as to ensure that a 3×3 array of 9 image pixels will cover approximately 40–60% of a bead's image after 10× magnification (as illustrated in FIG. **7**). Thus, in order to capture images of tiles of about 100 thousand 5 μm GMA beads, width (**210**) is selected to have a value of 1.7 mm. Length (**212**) is selected so that the flow chamber can hold from 1 to 10 tiles of about one hundred thousand 5 μm diameter beads each. The cross section (**220**) of inlet passage (**214**) matches that of the inlet tubing and gradually enlarges to match that of flow chamber (**201**) in the region of the planar cavity, i.e. the region holding the GMA beads on which analysis is performed. It is desirable to have a constant cross section through the planar cavity of flow chamber (**201**) to minimize the creation of non-uniform flow patterns, as might occur with sudden constrictions and/or expansions in cross section. Both body (**218**) and cover (**216**) of flow chamber (**201**) are glass. and the planar cavity and channels of body (**218**) are formed by standard chemical etching techniques. Cross section (**222**) of outlet passage (**224**) is selected to match the cross section of flow chamber (**201**) at dam (**202**).

The fluidics system of FIG. **5***a* which includes all valves, syringe pump (**500**), and Peltier block (**152**), is controlled by code written in LabVIEW 5.0 (National Instruments, Austin, Tex.) and run on a Compact Deskpro Pentium-based microprocessor, which is connected to the various components of the fluidics system by standard I/O circuit boards. Detection system (**114**) and overall control of the instrument is effected through a Sun Microsystems (Mountain View, Calif.) Sparcstation 5.

Three cycles of ligation, identification, and cleavage are carried out in flow chamber (**201**) to give the sequences of 12 nucleotides at the termini of each of approximately 500,000 cDNAs. That is, five tiles of GMA beads are analyzed in the following series of process steps:

1. Calibrate focal plane of GMA beads.
2. Hybridize decoder.
3. Autofocus on tile **1**.
4. Set focus to bead centers.
5. Collect fluorescent image.
6. Set focus to bead focal plane (scatter centers).
7. Collect image.
8. Repeat steps 4–7 for remaining tiles.
9. Wash.
10. Repeat steps 2–9 for remaining decoders.
11. Cleave encoded adaptor.
12. Wash.
13. Ligate top strand of next encoded adaptor.
14. Wash.
15. Repeat steps 13–14.
16. Kinase bottom strand of encoded adaptor.
17. Wash.
18. Ligate bottom strand of encoded adaptor.
19. Wash.
20. Repeat steps 2–9.
21. Repeat steps 11–19 for next encoded adaptor.

In steps 2–9, nucleotides of the cDNAs are identified by hybridizing tag complements to the encoded adaptors. Specifically hybridized tag complements are detected by exciting their fluorescent labels with illumination beam (**110**) from Xenon arc lamp (**126**). In step 13, encoded adaptors and T4 DNA ligase (Promega, Madison, Wis.) at about 0.75 units per μL are passed through the flow chamber at a flow rate of about 1–2 μL per minute for about 20–30 minutes at 16° C., after which wash of step 14 is executed by flowing, in succession, a solution of Pronase™ (Boehringer Mannheim, Indianapolis, Ind.), a salt wash solution, and an ethanol wash solution through the flow chamber, all with the same flow rate of 1–2 μL per minute and for durations of 15, 10, and 10 minutes, respectively. The salt wash solution is 150 mM NaCl and 10 mM Tris-HCl (pH 8.5), and the ethanol wash solution is 3:1 (v/v) solution of the salt wash solution and ethanol. The ligation and wash steps 13 and 14 are repeated once, after which the adaptors and the cDNAs are prepared for second strand ligation by passing T4 DNA kinase (New England Bioscience, Beverly, Mass.) at 7 units per μL through the flow chamber at 37° C. with a flow rate of 1–2 μL per minute for 15–20 minutes. Ligation of the second strand is carried out by flowing T4 DNA ligase (0.75 units per mL, Promega) through the flow chamber for 20–30 minutes at a rate of 1–2 μL per minute, followed by Pronase™ treatment and washing as described above. Tag complements at 25 nM concentration are passed through the flow chamber at a flow rate of 1–2 μL per minute for 10 minutes at 20° C., after which the fluorescent labels carried by the tag complements are illuminated and fluorescence is collected. The tag complements are melted from the encoded adaptors by passing NEB #2 restriction buffer with 3 mM $MgCl_2$ through the flow chamber at a flow rate of 1–2 μL per minute at 55° C. for 10 minutes. Encoded adaptors are cleaved from the cDNAs by passing Bbv I (New England Biosciences, Beverly, Mass.) at 1 unit/μL at a flow rate of 1–2 μL per minute for 20 minutes at 37° C., followed by Pronase™ treatment and washing, as described above.

SEQUENCE LISTING

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<223> OTHER INFORMATION: n = A,T,C or G

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ttttttttttt tttttttv 78

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<400> SEQUENCE: 2

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<400> SEQUENCE: 3

gatcagctgc tgcaaattt 19

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nnnctacagc tgcatccctc tacagcagcg 30

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<400> SEQUENCE: 18

nntntacagc tgcatccctg gtgaccgtag                                        30


<210> SEQ ID NO 19
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nnnttacagc tgcatccctc ccctgtcgga                                        30
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We claim:

1. A system for detecting a sequence of optical signals from each of a plurality of microparticles during a sequence of processing steps, the system comprising:

a planar array of uniformly sized spherical microparticles, wherein the coefficient of variation of the diameters of said microparticles is less than five percent;

an optical train effective to collect and focus the sequence of optical signals from the microparticles, and to record at least one optical characteristic of each microparticle which can be used to determine the approximate center of said microparticle;

an imaging device onto which said signals are focused, effective to generate and record a sequence of digital images of the microparticles, with sufficient resolution for individual microparticles to be distinguished; and

signal tracking means effective to correlate the optical signals from each of the microparticles in each of the sequence of digital images with said center of said microparticle.

2. The system of claim **1**, wherein the microparticles are substantially immobilized in said planar array.

3. The system of claim **1**, wherein said imaging device is a CCD (charge coupled device) camera.

4. The system of claim **1**, wherein said signal tracking means is effective to assign a plurality of pixels to each microparticle in each said image.

* * * * *

CERTIFICATE OF SERVICE

I hereby certify that on June 23, 2014, a true and correct copy of the foregoing Principal Brief of Appellant was filed and served via the Court's CM/ECF system. I further certify that I caused a copy to be served by electronic mail, and caused two copies to be served by Federal Express next-day delivery, upon the following:

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Dated: June 23, 2014

CERTIFICATE OF COMPLIANCE

1. This brief complies with the type-volume limitations of Fed. R. App. P. 28.1(e)(2)(A)(i) because this brief contains 11,565, excluding the parts of the brief exempted by Fed. R. App. 32(a)(7)(B)(iii) and Federal Circuit Rule 32(b).

2. This brief complies with the typeface requirements of Fed. R. App. P. 32(a)(5) and the type style requirements of Fed. R. App. P. 32(a)(6) because this brief has been prepared in a proportionally spaced typeface using Microsoft Word using 14-point Times New Roman font.

/s/ Christopher N. Sipes
Christopher N. Sipes

Dated: June 23, 2014